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SERUM DIAGNOSIS OF SYPHILIS

AND

THE BUTYRIC ACID TEST FOR SYPHILIS

BY

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14 ILLUSTRATIONS

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Printed by J. B. Lippincott Company
The Washington Square Press, Philadelphia, U. S. A.

To

DR. SIMON FLEXNER

THIS BOOKLET IS DEDICATED BY HIS PUPIL

THE AUTHOR

PREFACE

Since the application in 1906 by Wassermann and others of the Bordet-Gengou phenomenon of complement fixation to the diagnosis of syphilis, the large number of publications on the subject, while establishing the test as a diagnostic method of real value, have, unfortunately, aided the general medical reader little in gaining a clear conception of the principles involved; and the rapidly growing literature has become too voluminous to enable the average clinical laboratory worker to obtain exact data on the test, in its various forms.

The object of this book is, first, to give a brief yet adequate account of the principles of serum hæmolysis and of the behaviors of the combinations of antigens and antibodies towards hæmolysis, so essential for a proper understanding of the subject, discussing at some length the quantitative relationship of the factors playing a part in these phenomena, an aspect of the subject that has not perhaps received the consideration that it deserves; and, secondly, to give in detail the technic of Wassermann's method and of the method recommended by the author.

The author has endeavored to treat the subjects of this book in such a manner as to make it suitable for the use of practising physicians and students, and at

the same time with sufficient comprehensiveness to render it useful to laboratory workers.

The last chapter is a digression from the main subject, being devoted to a description of a chemical test for syphilis. The application of this test in the examination of cerebrospinal fluids is very simple and numerous trials have shown it to be an actual aid in the diagnosis of parasyphilitic affections of the central nervous system.

In the appendix the author has given an extensive bibliography, which should be of value to readers desiring further knowledge on the subject of the complement-fixation test for syphilis and certain closely allied problems of immunity, as well as on the cytological and chemical examination of cerebrospinal fluids, so useful in the diagnosis of parasyphilitic conditions.

The author wishes to express his indebtedness to Dr. Paul Lewis and Dr. David J. Kaliski for valuable assistance in the preparation of this book. He desires also to thank those who, by furnishing specimens for examination, facilitated completion of his methods, the early co-operation of Dr. Victor C. Pedersen and of Dr. J. W. Moore having been especially valuable.

HIDEYO NOGUCHI.

ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
NEW YORK, FEBRUARY, 1910.

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Serum Diagnosis of Syphilis

I.

SERUM HÆMOLYSIS.

The red blood-corpuscles of animals when put in contact with many different substances are so altered that their hæmoglobin is set free, the stromata also going into solution, as a rule. This phenomenon of solution is now generally known as *hæmolysis*. The substances which cause hæmolysis are said to be hæmolytic for the blood-corpuscles which they dissolve. Fresh blood-serum of many animal species is hæmolytic for the erythrocytes of some, but not all, other species. Hæmolysis by serum results from the coöperated (coördinated) action of two distinct serum principles or factors. The first is called *amboceptor*,[1] the second *complement*.[2] The latter is always present in all fresh sera: the former, on the other hand, is inconstantly so, frequently being absent from normal blood-serum. If erythrocytes are added to a serum which contains only amboceptor they absorb the am-

[1] Bordet's *substance sensibilisatrice*, and Metchnikoff's *fixateur*.
[2] Bordet's *alexin*, and Metchnikoff's *cytase*.

boceptor and retain it so firmly that even repeated washing with physiological salt solution cannot detach it from the corpuscles. The erythrocytes laden with amboceptor are said to have been *sensitized.* If complement be added to cells so prepared and washed they promptly dissolve. Erythrocytes do not absorb complement from a serum if there is no amboceptor present. The function of the amboceptor is to prepare or *sensitize* the erythrocytes for the attack of the complement, and that of the complement is to *dissolve* the sensitized erythrocytes. Amboceptor alone cannot dissolve the cells and complement likewise is ineffective if the cells are not prepared for its action. The particular constituent of the erythrocytes capable of uniting with the specific amboceptor is usually called the *receptor.*

A suspension of erythrocytes in physiological salt solution presents a bright orange-red, opaque appearance. The cells may be sedimented to the bottom of the receptacle, either by centrifugalization or by being allowed to stand for many hours, leaving above a clear colorless fluid. After hæmolysis, however, the suspension becomes deep pinkish red and transparent, being now a solution of hæmoglobin diffused out of the hæmolyzed erythrocytes. See Fig. 1.

These two essential hæmolytic components, amboceptor and complement, not only differ in their biological function, but also show differences in resistance to spontaneous deterioration, destruction by heat, and various other physical and chemical influences. Complement is labile and deteriorates gradu-

ally, disappearing from serum within a few weeks when kept on ice and within a few days when kept at room temperature. Exposure to a temperature of 55°–56° C. for one-half hour completely destroys the activity of complement. Amboceptor is much more

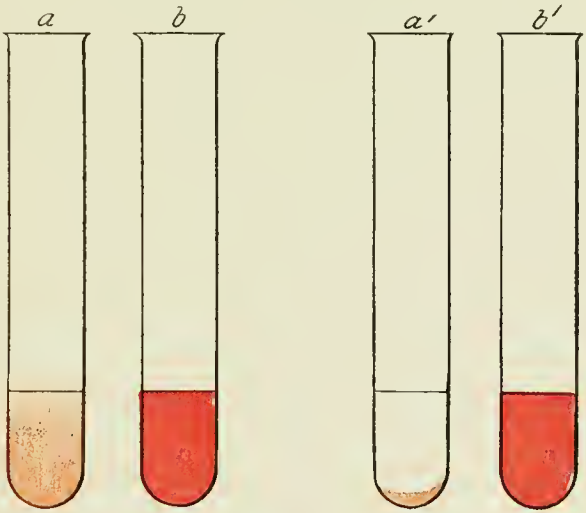


FIG. 1.—*a* shows a saline suspension of blood-corpuscles before hæmolysis; *b*, the same after hæmolysis. *a'* and *b'* present the appearance of *a* and *b* after sedimentation of corpuscles.

stable. It is usually still active in serum which has been kept for more than a year, and is not destroyed or markedly injured by exposure to a temperature of 55°–56° C. Serum is technically known as "fresh" or "active" serum within a day of its collection, while the complement is still fully active. The process of depriving a fresh serum of its complement by heat-

ing it to 55° C. is called *inactivation* of the serum. By an *inactivated serum* we mean one from which complement has been removed, but in which the amboceptor is left unchanged. If to an inactivated serum we add fresh serum (in a quantity inactive by itself because of the minute quantity or complete absence of its amboceptor) the mixture may produce hæmolysis in the presence of amboceptor in the inactive serum, because the complement destroyed by the process of inactivation is replaced by the complement of the fresh serum. This process of restoring the hæmolytic activity of an inactivated serum by the addition of fresh serum is known as *reactivation.* Fresh serum, thus used, inactive by itself, functionates by virtue of its complement-content, and is commonly called complement, being further designated by the name of the animal from which it is derived. Complement is always capable of reactivating the serum of the species from which it is derived, but not every complement can reactivate the sera of other species. In fact, there are only a few animals known whose complements can reactivate the inactivated sera of alien species. Otherwise stated, complement of one animal species is not identical in its action with that of another species. The interchangeability of complements, or the substitution of one for that of another species of animal, is only possible in a limited

number of instances. The complement of the guinea-pig is distinguished by an unusual ability to reactivate the sera of alien species and is consequently most often used when it is necessary to substitute the complement of one serum for that of another which has been inactivated or which has deteriorated.

The presence of amboceptor in blood-serum is much less constant than that of complement.

The amboceptor of any species acts always with the complement of the same species and less regularly, and to a limited extent only, with the complement of other species. In its relation to the red blood-corpuscles, however, the amboceptor is *specific:* that is, an amboceptor which can sensitize the erythrocytes of the rabbit, for example, to the action of complement cannot sensitize the erythrocytes of the sheep, dog, or any other animal. Amboceptors are named by prefixing "anti-" to the species-name of the cells against which they act. Thus an amboceptor active against sheep-corpuscles is known as antisheep amboceptor, and unites with the receptors of the former.

In any serum there are usually present many varieties of amboceptor. Thus in one serum there may be found amboceptors active against the blood-corpuscles of the sheep, dog, hen, rabbit, frog, man, etc. Different sera vary widely in the number of amboceptors present and in the relative quantity of

each. Among different specimens of serum from the same species the relative quantity of amboceptor can also vary considerably.

Amboceptors existing naturally in normal serum are known as *natural* or *normal amboceptors.* For example, human serum is frequently, though not always, quite hæmolytic for sheep's corpuscles, because it may contain natural antisheep amboceptor; but rabbit's serum, on the other hand, is incapable of hæmolysing human erythrocytes because of the absence in the rabbit of natural antihuman amboceptor.

As already stated, the serum of a given species may not contain amboceptors for the blood-corpuscles of some other species. If we select, for example, the rabbit, whose blood-serum contains no amboceptor for the erythrocytes of man, we may by repeated injections of the human erythrocytes into this animal produce specific amboceptor for human cells. This process of repeated injections with foreign cells (or any other suitable substance) is, in general, known as *immunization.* By a similar process we can also *increase* the amount of an amboceptor naturally present. Amboceptors thus artificially produced or increased are known as *immune amboceptors.* Amboceptors which act with the erythrocytes of the same species are known as *isohæmolytic amboceptors,* or, in short, *isohæmolysins.* It is extremely difficult to produce

an amboceptor for the erythrocytes of the same species by immunization.

The amount of complement is not perceptibly increased by immunization.

Summary.—We have learned that the fresh serum of an animal can hæmolyse the erythrocytes of another species or of as many species as its serum contains specific amboceptors for. The intensity of hæmolysis is, of course, proportional to the amount of each amboceptor present in the serum. The variety of natural amboceptors varies considerably according to the animal species.

The hæmolysis is caused by the specific, coördinated interaction of the amboceptor and complement of the serum on the erythrocytes. Complement is destroyed by heating to 55°–56° C. for half an hour, the serum being thereby inactivated. Amboceptor is not destroyed by this process. The hæmolytic activity of the serum can be restored by replacing the destroyed complement by the addition of complement contained in fresh serum of the same species or in that of a limited range of alien species whose complements are suitable for use. By immunization we can create a specific amboceptor for any kind of foreign erythrocytes. This immunization product possesses the same biological properties as the natural amboceptor and is called *immune* amboceptor.

II.

QUANTITATIVE FACTS ABOUT HÆMOLYSIS.

THE phenomenon of hæmolysis, as pointed out in the previous chapter, is dependent upon the action of complement and amboceptor upon erythrocytes. The first is contained in every fresh serum, the second may be contained in a given serum or can be artificially produced by immunization.

The hæmolytic activity of any serum is determined by mixing, in a series of test-tubes, a uniform quantity of a suspension of red corpuscles (erythrocytes) in a physiological salt solution [1] with graduated amounts of serum and bringing the whole to a constant volume by the addition of salt solution. The mixtures are placed at a temperature of 37° C. for a sufficiently long time to allow a complete reaction. The *titre* of the serum is usually expressed by the smallest amount of serum which is found to be necessary for the complete dissolution of all the corpuscles. With ordinary normal serum, complement is usually present in excess of the amount needed to activate all the amboceptor contained in the serum. The titre

[1] For hæmolytic work 0.85 per cent. (Ehrlich) to 0.9 per cent (Madsen) salt solution is universally employed. The writer uses the latter concentration.

of the serum in this case can be readily obtained by the above method; and it is dependent entirely on the amount of amboceptor present, for, as will be pointed out later, the activity of amboceptor is fully revealed only in the presence of an excess of complement. The conditions in dealing with an immune serum are more complicated. In it the normal complement-content is found associated with a great excess of amboceptor. A titration by the above method of simple dilution would disclose only the amount of complement in the serum, and a large, variable amount of amboceptor would remain inactive in the mixture, on account of the dilution to a minimum of complement. In order to arrive at the value of the serum in terms of its amboceptor another procedure must be adopted. There is placed in a series of test-tubes, as before, a definite and equal amount of corpuscle suspension and to each tube is then added an amount, also definite and equal, of a normal serum which has been found incapable in itself of causing hæmolysis (complement, see Chapter I). There is next added, in series, decreasing, graduated amounts of the immune serum whose native complement has been destroyed by inactivation. The titre of the immune serum is usually stated as the smallest amount of inactivated serum which produces complete hæmolysis in the presence of an excessive amount of a given suitable comple-

ment. It must be borne in mind that the titre of an immune serum will vary with the specific activity of the complement used. For example, an antihuman amboceptor prepared by treating a rabbit with human corpuscles is several times more active in the presence of a given amount of guinea-pig serum used as complement than it is when tested with the same quantity of human serum as complement. The strength of the immune serum expressed in terms of the smallest amount needed to produce complete hæmolysis will be quite different if 0.1 c.c. of complement has been used, from the value found if 0.05 c.c. has been used. *Within certain limits the quantitative relationship existing between the absolute amount of complement and amboceptor required to produce complete hæmolysis is such that an increase of one factor, say complement, permits the use of a less amount of the other factor, namely the amboceptor.*

In order to get uniform results with a reaction in which so many of the reagents are variable in activity, it is necessary to proceed in a definite order to fix standards of practical constancy. This order has been roughly outlined, but will bear restatement. A suitable suspension of erythrocytes is chosen and the amount of this suspension to be used arbitrarily fixed and kept constant. The total volume of the mixture is decided upon as another constant. A large and

uniform amount of complement is used in the first determination, an amount certainly in excess of that required for the complete activation of a moderate amount of amboceptor. With this amount of complement, in series, is combined decreasing quantities of amboceptor. The smallest amount of amboceptor required to produce complete hæmolysis is determined. This is not only, as was before stated, the titre or value of the immune serum, but it is best chosen for future work as the second fixed value in the reaction, the erythrocyte suspension being the first. In order to be sure that this is a constant value another test should now be made in which the quantity of amboceptor is still further reduced while the complement quantity is doubled. If all of the tubes in this series are not completely hæmolysed we may be certain that the complement quantity first chosen was large enough and that the amboceptor amount determined was actually the least amount which could under any circumstances produce complete hæmolysis of the corpuscle suspension used. This amount of amboceptor may be conveniently designated as one *amboceptor unit.*

Up to this point, it will be recalled, the amount of complement has been kept in excess. Now to each of a series of tubes containing the standard erythrocyte suspension one unit of amboceptor is added. If then in the series one puts decreasing amounts of comple-

ment the smallest amount of complement necessary to produce complete hæmolysis with one amboceptor unit will be determined. This amount is called one *complement* unit. The reaction could then be formulated as follows:

Standard erythrocyte suspension + 1 amboceptor unit + 1 complement unit = complete hæmolysis (time 2 hours at 37° C.).

As the erythrocyte suspension can be made of relatively constant value from day to day and as the amboceptor is stable over a period of months, the only actual variable from this point is the complement, and this can be standardized with a simple titration test.

The results, now, of varying the quantities of complement and amboceptor require the most careful consideration. If less than one unit of amboceptor is used hæmolysis will always be incomplete, even with more than one unit of complement. Likewise if with one amboceptor unit there is combined less than one unit of complement, hæmolysis cannot be complete. If with more than one unit of amboceptor there be used less than one unit of complement, hæmolysis may be complete or incomplete according to the relative amounts of each factor used. The complement may, of course, be reduced to such a degree that hæmolysis will be incomplete no matter

how much amboceptor is present. But in the presence of many units of amboceptor hæmolysis may be complete when but a small fraction of the complement unit is present. It is most important to note that a fraction of one unit of complement, too weak to produce any hæmolysis with one unit of amboceptor, can produce complete hæmolysis when combined with several amboceptor units. In other words, the activity of complement becomes steadily intensified or augmented by gradual increase of the number of amboceptor units until its maximum is reached. An amount of complement too small to cause complete hæmolysis in combination with one unit of amboceptor, may be sufficient to do so if two units of amboceptor are used, and an inactive quantity of complement with two units of amboceptor may no longer be inactive when four units or more are used, and so forth. Based upon this fundamental law of reduction in requirement of complement by increase of amboceptor to cause the same degree of hæmolysis, we can easily see that hæmolysis is merely the relative expression of the combined action of amboceptor and complement and is not the absolute indication of the amount of the hæmolytic components present in a fluid. The same amount of hæmolysis can be produced by one unit of complement and one unit of amboceptor or by 20 units of amboceptor and 0.1 unit

of complement, or any other appropriate combinations of these two components. Unless the amount of amboceptor used is the same in any two sets of hæmolytic experiments the amount of complement acting in these two sets cannot be estimated by comparing the degree of hæmolysis. In order to calculate the exact amount of complement needed to produce a given amount of hæmolysis it is necessary to know the amount of amboceptor used, because the activity of complement is different according to the amount of amboceptor present. It is therefore erroneous to conclude that the degree of hæmolysis produced by one unit of amboceptor and by twenty units of amboceptor is the work of the same amount of complement. These facts are graphically shown in the accompanying diagrams (Figs. 2, 3 and 4).

For any given relative proportion the result can be determined experimentally, of course, and the reaction follows certain laws with sufficient regularity so that the result can be calculated. But it is exactly for the purpose of avoiding the complexities and uncertainties introduced by this quantitative variation that we take such pains to get fixed standards, and the more closely these standards are adhered to the simpler will be the conditions and the more easy and accurate the interpretation of the results.

We will now consider the fate of amboceptor and

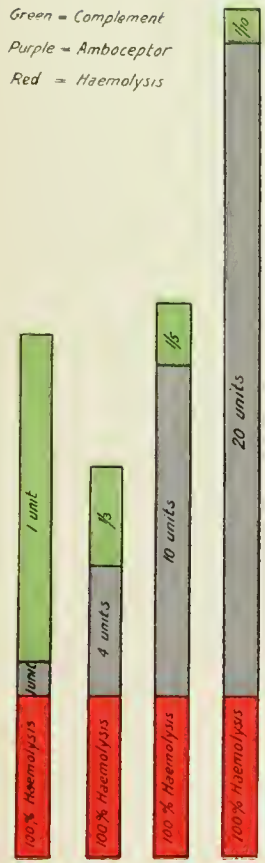


FIG. 2.

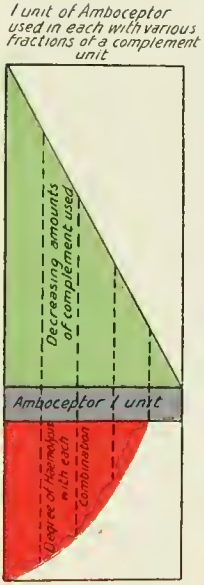


FIG. 3.

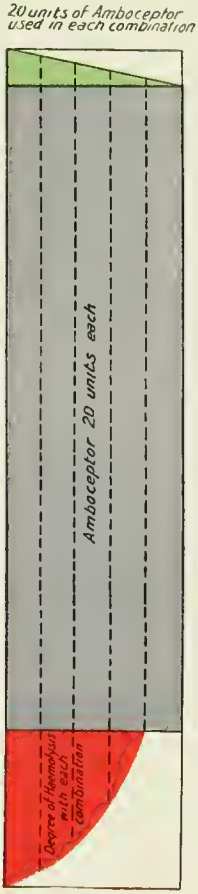


FIG. 4.

complement after the reaction has run to completion.

It has been experimentally determined that within certain quantitative limits they have disappeared from the mixture, having been used up in producing hæmolysis. The limits within which this disappearance is complete are roughly as follows: Where one unit of amboceptor has been used with one unit of complement the disappearance of both will be complete. When with one unit of amboceptor more than one unit of complement is used, an excess of complement will be found still present in the fluid after the reaction is accomplished. The same applies to the combination of one unit of complement with less than one unit of amboceptor, in which case hæmolysis will be incomplete. When with one unit of complement there is combined more than one unit of amboceptor the excess of amboceptor shortens the time necessary for complete hæmolysis. The corpuscles being capable of absorbing more amboceptor than is necessary for complete hæmolysis, most of the excess is taken up by them in this way. If the excess is very large (more than the corpuscle mass can absorb), it may be found free in the fluid after the reaction is ended. If more than one unit of amboceptor and of complement are used an excess of both may be found in the fluid after the hæmolysis is complete.

Enough has been stated to show the reader that

we are dealing with a complex reaction whose factors have a definite relationship to one another which can be accurately determined. If the co-relative values of complement and amboceptor are carefully determined and rigidly adhered to according to the outline given, precise and constant results can be obtained in the practical application of the reaction, which will be developed in the following pages. If in using the reaction these quantitative steps are not observed one cannot hope for useful or accurate results.

III.

ANTIGENS AND ANTIBODIES.

I HAVE already stated that by repeated injections of erythrocytes of one animal into an alien species we can produce in the latter an amboceptor having a specific affinity towards these erythrocytes. A similar phenomenon is observed when bacteria are injected. We call amboceptors for the blood-corpuscles *hæmolytic amboceptors* and those for the bacteria *bacteriolytic amboceptors*. Their general characteristics are the same, differing only in that they have a specific affinity towards the substances which gave rise to them. Bacteria when treated with specific bacteriolytic amboceptor absorb the latter and become sensitive to the dissolving action of complement. This phenomenon of dissolution is called *bacteriolysis* and in its mechanism is comparable to that of *hæmolysis* in every respect.

It is found that when various unorganized protein substances are injected into an animal they elicit a similar response, giving rise to various specific immune substances or reaction products. Egg albumin and alien blood-serum, for example, when injected give rise to specific *precipitins*. Serum containing a

specific precipitin when mixed with a solution of the protein which was injected in order to develop it causes a precipitate to form. Mixed with any other protein solution the precipitation does not occur. The substances—erythrocytes, bacteria, or unorganized foreign protein—which when injected produce corresponding specific reaction products—are called *antigens.* The reaction products of whatever kind which are produced by the animal are called *antibodies.* Immune hæmolytic amboceptors, bacteriolytic amboceptors, and precipitins are therefore antibodies produced by injecting as antigens erythrocytes, bacteria, or unorganized proteins.

The most striking characteristic of the antibodies is their specific relationship with the corresponding antigens. Antigen *A* gives rise to antibody *A,* and antibody *A* reacts outside the body with antigen *A* and with no other. It is scarcely necessary to recall the well-known fact that the serum of a typhoid patient (containing typhoid agglutinin) agglutinates only the typhoid bacillus and none of the closely related intestinal micro-organisms. The hæmolytic amboceptor for human erythrocytes acts only with those erythrocytes, and not with the cells of any other species. Similarly, as was before stated, the precipitin obtained by injecting man, monkey, or rabbit serum causes precipitation only with the particular serum

used to produce it. The few exceptions to this general rule may be neglected in our present discussion.[1]

Taking into account this quality of specificity it will be readily seen that if we have an unknown antibody to deal with we can identify it by putting it in contact with a number of different antigens under favorable conditions and noting the one with which it reacts. With a known antibody the character of an unknown antigen can likewise be determined. This *direct method* of recognizing unknown antibody has been used in a number of different ways in recent years. Some instances, such as the Widal reaction in typhoid fever, are known to every one. Another important test dependent on this principle is the precipitation method for determining the species of animal from which a specimen of blood of unknown origin may have come. Artificial antibodies are produced by treating animals—rabbits, for example—with the blood-serum of a number of different animal species. The unknown blood is dissolved in physiological salt solution and put in contact with this series of known antibodies (precipitins). That antibody with which a precipitate is formed must be, according to the law

[1] For example, I found that antigoat serum (rabbit) contains precipitins not only for goat serum but also for sheep and ox serums in smaller quantity, while anti-ox serum (rabbit) contains precipitin only for ox serum. Antisheep serum contains precipitin for sheep and goat serums, but not for ox serum. This phenomenon is comparable to group reaction of agglutination.

of specificity, antibody prepared with serum of the same species as that from which the specimen in question was derived.

The effects we have so far considered have all been the direct and essential manifestation of reactions between antigen and antibody with or without the associated action of complement as the case may be.

Studying the phenomena of interaction of antigen and antibody in general, we will discover a peculiar relation which exists between the combination of antigen-antibody and complement. We have already seen that the erythrocytes (antigen) acted on by amboceptor (antibody) become so altered as to absorb complement and undergo hæmolysis. We have also learned that bacteria (antigen), after having been acted on by amboceptor (antibody), take up complement and become dissolved by the complement. Now a question arises as to whether unorganized antigens display the same characteristics as the antigens in previous instances when brought together with their specific antibodies. Through experiment this was found to be the case. Thus when a precipitable antigen (precipitinogen) is brought in contact with its specific precipitin it not only forms a visible precipitate, but also becomes capable of absorbing or fixing complement. If to a mixture of a precipitable antigen—for example, blood-serum or egg albumin—

and its precipitin, complement be added during or after the reaction period, and if the mixture be subsequently tested for the presence of complement by adding erythrocytes and their specific hæmolytic amboceptor to the mixture, it is found that the complement has disappeared; that is, hæmolysis does not take place. This phenomenon of disappearance of complement in the mixture of antigen and antibody is now generally called *fixation of complement*. Sometimes it is called *deviation of complement* on account of the fact that the complement has been deviated by the combination of antigen and antibody and prevented from participating in the hæmolytic process. These facts were first brought out by the investigations of Bordet and Gengou and the reaction is known accordingly as the Bordet-Gengou phenomenon of complement fixation.

It is found that the mixture of antigen and antibody can fix complement in a dilution in which a visible precipitation is no more obtainable. In other words, the fixation phenomenon is capable of indicating the existence of the antigen-antibody reaction beyond the delicacy that a visible precipitation can attain.

Concerning the phenomenon of complement fixation, it would be well to point out here that the sera (complement) of various animals present marked dif-

ferences in regard to this property. Some complements are easily fixed in the presence of the antigen-antibody combination, others slightly or not at all. While the serum of an animal may possess the property of reactivating the hæmolytic amboceptor of an inactivated serum, yet the serum of this species may possess little or no fixation property. This fact becomes of great importance, as will be seen later, in utilizing the complement fixation phenomenon in diagnosis.

Working with three different antigen-antibody combinations, namely, precipitates formed by mixing human serum, egg albumin, and meningococcus extract with their specific precipitins, the writer has found that the fixability of the sera (complement) of various animals differs widely. Guinea-pigs' complement is most easily fixed, goats' complement being hardly fixed at all. The complements of man, horse, ox, sheep, and rabbit are fixed in intermediate and varying degrees to those mentioned before. In performing these experiments two different amboceptors (both specific for human corpuscles) produced in rabbits and goat were used. Rabbits' complement possessed, as a rule, the best reactivating property for the rabbits' amboceptor, but the complements of sheep, pig, and ox seemed often devoid of this property. The complements of man, goat, and horse are weakly reactivating for this amboceptor. The

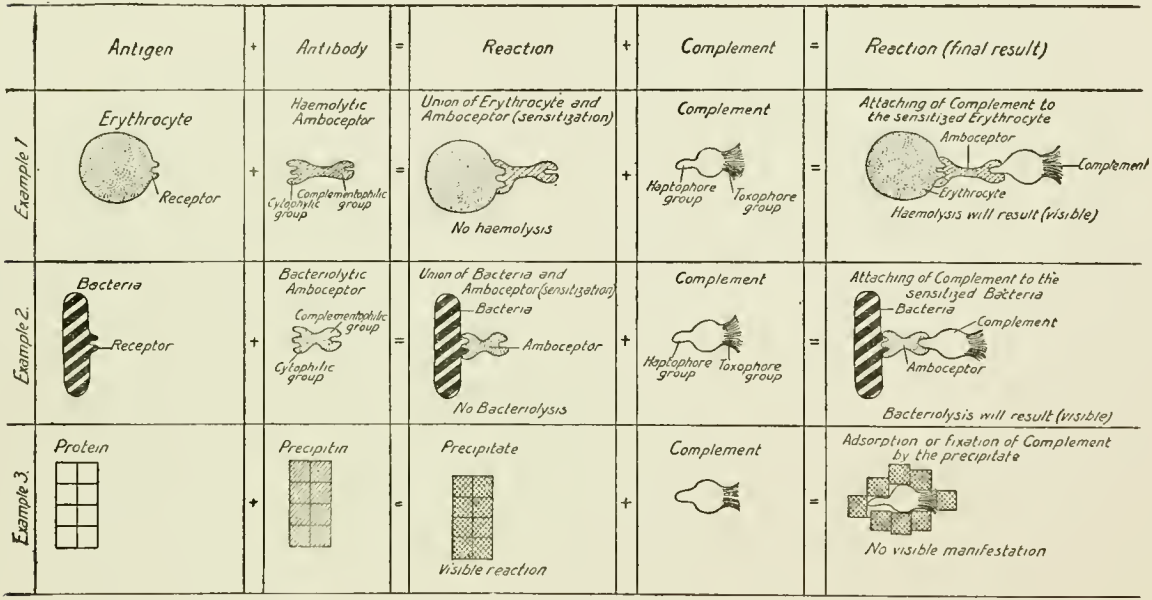


FIG. 5.—Shows the components required and the steps of reactions followed in producing hæmolysis, bacteriolysis, and adsorption or fixation of complement by a precipitate.

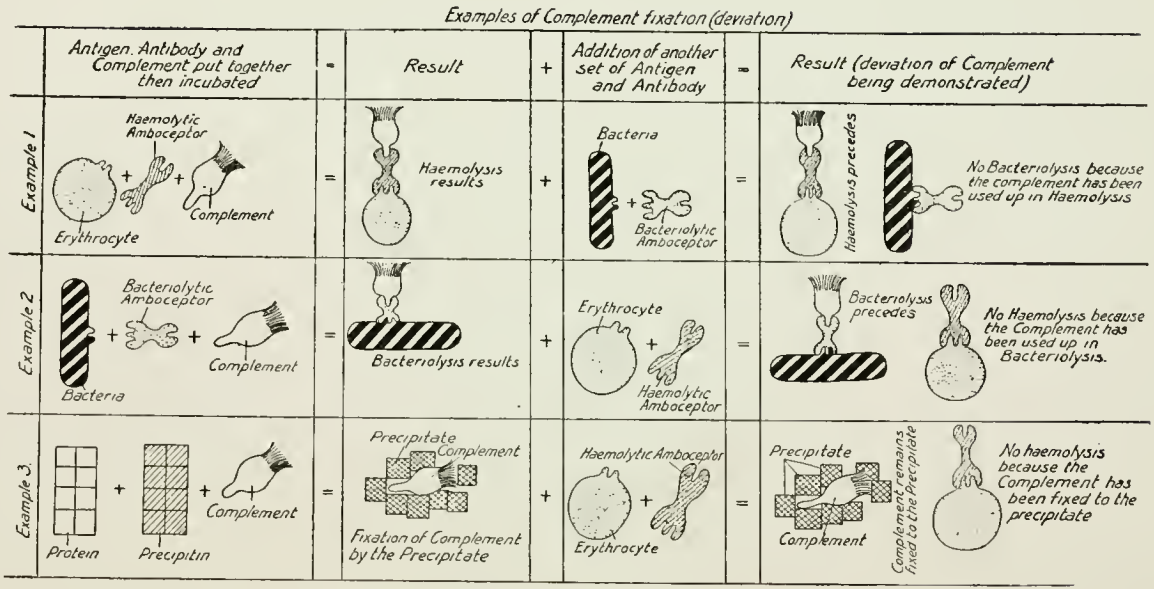


FIG. 6.—Illustrating the phenomenon of Bordet and Gengou. The serum reaction of Wassermann for syphilis is analogous to Example 3, except that the lipoidal substance corresponds with protein and syphilitic serum with precipitin.

amboceptor from goats could be reactivated by all of the above sera excepting that of pigs, although goats' serum was most effective and guinea-pigs' somewhat less so.

We have also seen the mode of detecting, by means of the precipitation reaction, an unknown antigen or antibody by direct observation. Now antigen can also be detected, by indirect observation, through the employment of the complement-fixation reaction.

To illustrate the mechanism of the Bordet-Gengou phenomena I introduce here some schematic illustrations based upon the well-known side-chain theory of Ehrlich. Fig. 5 shows three different combinations of antigens and antibodies, each capable of grasping or attracting complement.

Fig. 6 illustrates the deviation of complement by one combination of antigen and antibody preventing the complement from taking part in another reaction after such fixation.

The application of this indirect method of determining the presence of a specific reaction between antigen and antibody has been extensively applied to various infectious diseases with more or less success. The details of the application of this principle to syphilis will be developed in the following pages.

IV.

THE APPLICATION OF THE INDIRECT METHOD OF DETERMINING ANTI-BODIES TO THE DIAGNOSIS OF SYPHILIS.

We have so far developed the fact that combinations of antigen and antibody which do not require complements to complete their characteristic reaction may still bind complement and prevent its taking part in other reactions. We have also noted that the property of fixing complement may be exerted by quantities of antigen and antibody which are too small to give rise to the characteristic reaction of such a combination, namely, visible precipitation. From this it was but a short step to the conception that there might be a combination of antigen and antibody for which we are acquainted with no characteristic direct manifestation but which could still exert a fixing effect on complement.

From clinical studies it has long been known that syphilis is an infectious disease which in running its course produces a specific immunity. That an immunity is developed means, presumably, that antibodies against the infectious agent are produced in the subject at some stage of the process. As the infectious agent has not up to the present time been

cultivated or by other means separated in any quantity from the diseased tissues, it is impossible to determine the presence of antibodies by the direct observation of such a reaction as bacteriolysis or agglutination. Moreover, even though we accept the *Treponema* (*Spirochæta*) *pallidum* as the cause of the disease, the detection of its presence is not to be relied upon as our only means of diagnosis. In the late manifestations of the disease, at a time when it is still infectious and still amenable to specific treatment, the treponemata are present in such small numbers as to be most difficult of detection. In exactly these cases a measure of immunity may be supposed to have developed and specific antibodies to have been formed.

The idea, then, was to take syphilitic tissues at a stage when the treponemata were most numerous and use this as the known antigen. Tests against this known antigen with blood-serum of other patients might, it could be hoped, reveal the presence of antibody. As has been said, the direct method of observation had nothing to offer and the indirect method was tried from the first.

The earliest publication on this indirect method of detecting the syphilitic antibody is that by Wassermann, Neisser, and Bruck on May 10, 1906, and the next article is that by Ladislaus Detre on May 24 of the same year. These investigators were working

on the same subject independently during the same period of time and obtained exactly the same results.

The technic employed by Wassermann, Neisser, and Bruck, on the one hand, and by Detre, on the other, was almost identical except in small details and will be stated here in a general way.

Extracts of syphilitic tissues in the active stages of the disease were made and used as antigen. Wassermann, Neisser, and Bruck used the liver of a congenitally syphilitic fœtus, and Detre employed chiefly condylomata for this purpose. Serum of known syphilitics, inactivated at 56° C., was used as antibody. To this combination complement was added. So far there was no visible change. If after a short time a quantity of immune hæmolytic amboceptor was added to the mixture, and then the cells for which this amboceptor was developed, no hæmolysis took place. It can be shown by suitable experiments that the failure of the erythrocytes to dissolve is not due to any change in the cells. Neither is it due to interference with the amboceptor. The complement has been prevented from acting, has been fixed, or deviated. If in place of the blood-serum of a known syphilitic there had been used as unknown antibody the blood of a person known never to have had syphilis, hæmolysis would occur, because the complement was not interfered with. The erythrocytes used by Wassermann,

Neisser, and Bruck were those of the sheep, and those used by Detre were from the horse. The amboceptors employed were of course an antisheep serum in the first instance and an antihorse serum in the second, both being derived from rabbits immunized with the erythrocytes of the corresponding animals.

In the beginning it was supposed that the antigen also was specific; that is, that the serum of the syphilitic patient would only fix complement in the presence of extracts of syphilitic tissues. By experiments of Landsteiner, Müller, and Pötzl; Levaditi; Weil and Braun; of Meier; and also of the writer, it has, however, been determined that when such a serum is combined with the alcoholic extracts of certain normal organs or with a preparation of crude tissue-lecithin complement is also fixed. Because they are easier to obtain such nonspecific extracts are now commonly used as antigens in this reaction. When the diseased tissues are used as the source of antigen the liver of a syphilitic fœtus is commonly chosen.

Summary.—It has been found on the basis of thousands of comparative tests that if the blood-serum of a patient suffering from syphilis be mixed in the presence of complement with extracts of syphilitic livers, with alcoholic extracts of certain normal organs, or with crude tissue-lecithin preparations, the complement will be fixed and prevented from taking

part in a subsequent hæmolytic reaction. Starting with the extracts given and mixing with them, in the presence of complement, serum of unknown origin, if complement be fixed it can be stated with assurance (with certain reservations to be discussed later) that the unknown serum was derived from a case of syphilis.

To those who have carefully considered the quantitative relationship of the factors in a simple reaction with a hæmolytic serum as presented in the first chapters of this book, it will at once be apparent that in a test in which the hæmolytic reaction is to be used as an indicator of a second reaction not otherwise visible, the quantitative values of all the factors must be accurately determined and rigidly adhered to if the results are to be relied upon. The following chapter is devoted to the consideration of the quantitative relationship of these factors and to certain other precautionary measures which must be observed.

V.

QUANTITATIVE RELATIONS OF THE FACTORS IN THE SERUM DIAGNOSIS OF SYPHILIS.

It will be recalled that in the complement-fixation reaction there enter into operation five distinct and essential factors. Enumerated, they are: complement, syphilitic antigen, syphilitic antibody, erythrocytes, and hæmolytic amboceptor. Two of these factors are antigens, the erythrocyte and the organic extract; two of them are antibodies, the hæmolytic amboceptor and the syphilitic antibody. To avoid confusion in the discussion of the reaction it has been customary to group these factors according to their functions as follows: the erythrocytes, the hæmolytic amboceptor, and the complement are collectively referred to as the *hæmolytic system.* Speaking of the first two factors of the hæmolytic system it is customary to avoid the terms antigen and antibody and speak of erythrocytes and amboceptor. When in connection with the test we refer to antigen and antibody, we mean only the two factors outside the hæmolytic system, the syphilitic antigen and the antibody present or absent in the serum to be tested. In principle any hæmolytic system can be used as an

indicator for the test, provided the complement used is sensitive to fixation.

Whatever system be chosen the relation of amboceptor, complement, and erythrocytes as outlined in the second chapter must be observed. That is, one must work with a suspension of erythrocytes of definite value, the amboceptor must be carefully titrated with respect to that erythrocyte suspension, and with respect to the complement employed. Then definite amounts of amboceptor must be used, and the quantity of complement to be employed must be constant, and so adjusted as to act with the quantity of amboceptor and erythrocyte suspension determined upon. If, for example, we use in the hæmolytic system one amboceptor unit, at least one complement unit must be combined with this in order to obtain complete hæmolysis. If less than one complement unit were added to the antigen-antibody mixture in the fixation test, then hæmolysis would certainly be incomplete and one might imagine that complement was fixed when it was merely deficient in quantity, from the beginning. If many amboceptor units are used hæmolysis may be complete in the presence of much less than one unit of complement. Theoretically the test for fixation of complement might be made much more delicate by uniting two units of amboceptor with only a fraction of one

unit of complement. In practice, however, there are certain extrinsic factors which may interfere with the action of small amounts of complement, and it is therefore not safe to use minimal quantities. These factors will be more carefully and fully considered later.

A more common quantitative error is the following: Suppose that the antigen-antibody combination in any given test is capable of binding one and one-half units of complement. The one-half unit of complement remaining, two units being used in the test, would be incapable of completing hæmolysis with the amount of amboceptor which should be added, but with a high multiple of that amount could easily do so. We would then obscure, by an excess of amboceptor a positive reaction. If, on the other hand, with a small amount of syphilitic antigen and antibody we use an excessive amount of complement, the antigen-antibody combination may bind complement up to its capacity and still leave a sufficient quantity to act in conjunction with the amboceptor to produce hæmolysis. We would then again have a false negative instead of a positive reaction.

It was stated that certain factors not directly involved in the reaction might interfere with it if small amounts of complement are used. There are at times substances in the extracts other than the specific an-

tigen which prevent complement from acting. They are commonly known as anticomplementary substances. Anticomplementary substances may also exist in the serum to be tested. In order to guard against error from this source when making the test with preparations with more or less anticomplementary action it is necessary to add antigen alone, and the test serum alone, to the hæmolytic system in somewhat larger quantity than they are to be used combined in the test. Human serum may contain natural hæmolytic amboceptors for the erythrocytes in use when the erythrocytes belong to alien bloods. Thus if we add one unit of *immune* amboceptor to a mixture already containing six units of *natural* amboceptor, it would produce exactly the same discrepancy in result as though the immune amboceptor were added in excess. This source of error is to be avoided by choosing a hæmolytic system for which human serum contains no natural hæmolytic amboceptors. There may also be hæmolytic substances in the antigen preparations. These must be tested for each time when dealing with unknown or freshly prepared extracts.

Coming now to the exact quantities to be used; the determination of the strength of amboceptor and complement has been described in detail (see Chapter II). It is customary to use a slight excess of each,

usually two units. This makes the reaction somewhat less delicate, but allows a margin for error due to anticomplementary substances and in the long run makes the test more reliable. The amount of serum to be tested for its antibody content must be large enough to bind all complement in the presence of sufficient antigen if the serum be from a known syphilitic case. The proper quantity of the serum to be used depends, therefore, upon the quantity of the complement used in the hæmolytic system. It is obviously of advantage to construct a system in which as small an amount as possible of patient's serum can be employed without diminishing the delicacy or reliability of the reaction.

VI.

VARIOUS FORMS OF THE COMPLEMENT FIXATION TEST AS APPLIED TO THE SERUM DIAGNOSIS OF SYPHILIS.

As was pointed out in the last chapter, it is theoretically possible to use almost any hæmolytic system to test the binding power of antigen-antibody combination for complement. At least nine such systems have been used up to the present time. These systems can be divided into two groups according to whether foreign or human corpuscles are used as the hæmolytic indicator. A brief critical review of these will perhaps make clearer the principles of the test.

Wassermann, Neisser, and Bruck use sheep blood-corpuscles, an immune hæmolytic amboceptor made by immunizing a rabbit with sheep's erythrocytes, and guinea-pig complement. In making the test the syphilitic serum is inactivated. The quantity used is 0.1 c.c. to 0.2 c.c. for each test-tube. Two units of the amboceptor and 0.1 c.c. of guinea-pig's complement are used against 1.0 c.c. of a 5 per cent. suspension of the washed sheep-corpuscles. The resultant volume of the whole mixture is brought up uniformly to 5 c.c. There is but one large factor of

error and that operates as follows: There is in human serum a variable amount of natural antisheep amboceptor. In the cases in which such an amboceptor is present in appreciable quantity it serves to increase the total effective amboceptor in the mixture. This, according to the relationship existing between the amount of amboceptor and complement respectively required for complete hæmolysis, tends always to make hæmolysis complete even when antigen-antibody has fixed a considerable amount of the complement (Figs. 2, 3, and 4, and references to previous chapters). It is also possible that the complement which has been completely fixed by a moderate amount of antigen-antibody combination may become once more detached if the amount of the hæmolytic amboceptor introduced be very large, and produce complete hæmolysis. As the occurrence of hæmolysis means a negative reaction, or absence of syphilitic antibody, the error in this case is always in the direction of throwing sera with smaller amounts of syphilitic antibody into the negative class. If an error in diagnosis is inevitable it is of course safer to have it in this direction; but, as will be pointed out later, this source of error can be avoided by a change in the hæmolytic system.

Bauer in his test relies entirely for amboceptor upon the natural antisheep amboceptor in human

serum, which, as has been pointed out, is a source of error in Wassermann's system. This is merely a makeshift and does not eliminate the error, because amboceptor is not always naturally present and when present varies greatly in quantity. For this reason the test is unreliable, because of oversensitiveness, since, as pointed out, amboceptor may not be present at all or only, as may happen, in a fraction of one unit.

Hecht relies not only on the natural antisheep amboceptor of human serum but also on human complement. It will be seen at once that if this were a system whose factors were regular in quantity, it would be much simpler in practice than Wassermann's system. All that would be necessary would be to add antigen to the patient's own serum, which would contain complement and antibody to be detected, and amboceptor. After a period of incubation sheep erythrocytes would be added and the test read " positive " or " negative " after another period of incubation. However, not only is the amboceptor a factor varying from zero to ten units or more, but human complement is far less regular in amount and activity than is guinea-pig's complement. Further, the test would have to be done with fresh serum or the complement would surely be reduced or totally lost by spontaneous deterioration. There is not in this

system a *direct way* of testing the anticomplementary action of antigen alone.

M. Stern proposed a system in which there were added a few units of immune antisheep amboceptor to the fresh serum to be tested, utilizing the complement of the patient's serum. This still retains all the defects inherent in the use of unknown and often excessive amount of the hæmolytic amboceptor, and makes it impossible to test a specimen that has been kept a few days after collection.[1]

Detre, and Detre and Brezovsky, used horse corpuscles, an immune antihorse hæmolytic amboceptor derived from a rabbit injected with these erythrocytes, and rabbit's complement. As human serum contains natural antihorse amboceptor to about the same degree and frequency as antisheep amboceptor, this system is no more reliable than Wassermann's system. Further, the reagents are difficult to procure.

Boas advocates a system similar to the Wassermann system, using an antigoat amboceptor produced in rabbits. It has defects similar to the original Wassermann test.

[1] According to my recent investigations certain proteins, such as pepton, albumoses, nucleoproteins, and certain peptids can often produce complement-fixation when mixed with unheated human serum, closely resembling specific fixation. For this reason no active serum should be used for the test with aqueous or even alcoholic extracts of liver, especially of macerated organs. Pure lipoids free from above mentioned substances do not give this false fixation with active serum (Noguchi).

Browning used an anti-ox amboceptor produced in rabbits, and claims that human serum does not contain a disturbing excess of anti-ox amboceptor.

Tschernogubow proposed a system in which the natural amboceptor and complement of human serum are utilized against guinea-pig corpuscles. This system neglects entirely the quantitative phases of hæmolysis and is unworthy of consideration.

These seven different systems may be considered as of one general order, having in common the use of erythrocytes of animals for which human serum contains an unknown and irregular quantity of natural hæmolytic amboceptors. Except the system of Wassermann, Neisser, and Bruck, of Detre, of Boas, and of Browning, none of these is to be recommended for diagnostic purposes.

Tschernogubow, in an article published several months before his second system, discussed with the foregoing group, also proposed a system, which he has since abandoned, with quite a different set of factors. In general, it has much in common with the system put forward at about the same time by the author. Both systems use human erythrocytes and antihuman hæmolytic amboceptor, but the source of complement and the manner of conducting the test are altogether different. Tschernogubow collects the patient's blood (not serum) in saline solution

in such dilution that clotting is temporarily prevented. This suspension when fresh contains complement, erythrocytes, and, if present, the syphilitic antibody. When the antigen is added the antibody unites with it and the combination fixes complement. The antihuman amboceptor is added later, when hæmolysis occurs in case the complement has not been fixed. This system shows four sources of error.[1] The amount of complement in human serum is irregular and its activity is weak in relation to antihuman hæmolytic amboceptor. The complement and erythrocytes deteriorate rather rapidly, hence no examination can be made of old specimens. The complement cannot be put in contact with antigen alone and consequently it is impossible to decide if the antigen is or is not inherently anticomplementary by a direct test (Chapter V). Strangely enough, Tschernogubow did not state the source of his antihuman amboceptor. He does say that 0.25 c.c. was added to each tube. This is nearly 125 times the amount of amboceptor I use in combination with guinea-pig complement. It is probable that in this way he overcomes, in a measure, the variations in the amount of human complement present. Still, the

[1] A fifth source of error has since been discovered by me, that is, the use of active serum in combination with aqueous extracts of liver renders the test nonspecific. This objection applies to any other system using active serum and aqueous or even alcoholic extracts of macerated livers.

uncertainty in this respect, the impossibility of separating the factors in the system for control, and, lastly, the necessity of making the test very soon after the collection of the blood in order that complement may be active and the erythrocytes intact, leave abundant room for improvement and render the system as outlined too unreliable for general use.

After considerable experience with the Wassermann test in its original form, using both the aqueous and the alcoholic extracts as antigen, I became convinced of its value in the diagnosis of syphilitic and parasyphilitic conditions. I felt, however, that if the reaction was to come into general use it would have to be greatly simplified. In attempting such a simplification it was essential that nothing making for accuracy be sacrificed. It was thought that it would be of immense advantage to eliminate, if possible, the error due to the irregular presence of natural antisheep amboceptor in human serum. The directions in which simplification was most needed will be briefly alluded to. The Wassermann test demanded the use of fresh washed sheep's corpuscles each time a test was to be made. Persons far removed from a large abattoir would have difficulty in obtaining sheep's blood. Washing the corpuscles was essential, and required a good centrifuge. The serum must be inactivated, demanding care and a water-bath. *Fresh*

guinea-pig serum for complement must always be on hand. As many investigators would only occasionally use such serum this might demand the sacrifice of a guinea-pig for each separate test. The measuring and graduation of dosage of the liquid preparations require a full equipment of laboratory glassware. On the whole, the labor was so great that even in a fully equipped laboratory a man who proposed to carry out the test as a routine procedure could do little else; and outside such a laboratory the performance of the test was not to be thought of. In the method which eliminates these difficulties, a dilute suspension of human erythrocytes is used. This is readily prepared at any time by pricking the finger and allowing the blood to drop into physiological salt solution. It has been found possible to prepare antigen, antihuman hæmolytic amboceptor, and guinea-pig complement, in the form of reagent papers, which remain stable so long as kept perfectly dry. The factors, it will be noted, are still separate and distinct, as in the Wassermann system. By using an antihuman hæmolytic system the variable antisheep amboceptor of human serum is eliminated as a disturbing factor. The advantage, in point of regularity and uniformity gained by the use of guinea-pig complement is retained without any sacrifice in accuracy. And, further, the test can be put within reach of any

TABLE 1.

	Systems	Hæmolytic system			Patient's serum	Antigen
		Complement	Amboceptor	Blood-corpuscles		
GROUP A	Wassermann, Neisser, and Bruck.	Guinea-pig's fresh serum, known definite quantity, 0.1 c.c.	Antisheep amboceptor. From two sources: that which is present normally in human serum, of variable quantity, and that which is added in form of immune amboceptor derived from rabbit (2 units). The total sum of the amboceptor is therefore variable and unknown.	Sheep's washed corpuscles, known definite quantity. 1 c.c. of 5 per cent. suspension.	Inactivated before use. 0.1–0.2 c.c., requiring 5 c.c. of blood.	Liquid preparation, known adequate quantity. Aqueous extract of syphilitic fetal liver.
	Bauer	Do.	Antisheep amboceptor. From one source, namely in the serum to be tested. No immune amboceptor added. Thus the amount of the amboceptor used in the test is variable and unknown.	Do.	Do.	Do.
	Stern	Like Hecht's system.	Like Wassermann's system, viz., uses natural and immune antisheep amboceptors.	Do.	Like Hecht's system.	Like Hecht's system.

GROUP A—*Continued*	Hecht	Utilizes human complement as naturally present in the fresh serum. Old specimens cannot be tested. Complement and syphilitic antibody exist inseparably in one serum if the latter is present at all. The quantity is rather variable.	As in Bauer's system	....Do	Tested only in perfectly fresh state without inactivation. Impractical on account of inability to examine specimens several days old. Quantity definite but quite large.	Like the original Wassermann there is no direct way of testing its anticomplementary power on complement independent of syphilitic antibody. There is danger of obtaining nonspecific reaction.
	Detre	Rabbit's fresh serum, known definite quantity, 0.2 c.c.	Antihorse amboceptor from immunized rabbit, 2 units.	Washed horse-corpuscles.	Inactivated before use. Known quantity, 0.1–0.2 c.c.	Known adequate quantity in fluid form.
	Boas	Like Wassermann	Antigoat amboceptor, 2½ units.	Goat corpuscles 1 c.c. 5 per cent. suspension.	....Do	Alcoholic extract of human heart.
	Browning	....Do	Anti-ox amboceptor	Ox corpuscles	....Do	....Do.
	Tschernogubow.	Human complement	Antiguinea-pig amboceptor found in patient's serum.	Washed guinea-pig's corpuscles.	To be tested while perfectly fresh.	Aqueous extract of syphilitic liver. Danger of nonspecific reaction.
GROUP B	Tschernogubow.	Human complement as present in the patient's blood. Variable. The same objections as in the case of Hecht's and Stern's systems.	Antihuman amboceptor. Source unstated. An enormous quantity used, hence uneconomical.	Human corpuscles, not washed and containing fibrin ferment.	Tested only while perfectly fresh, otherwise impossible. Quantity not adjustable.	Dried syphilitic liver extracted before use. Danger of nonspecific reaction.
	Noguchi	Guinea-pig's serum fresh. Definite quantity (2 units), usually 0.04 c.c.	Antihuman amboceptor from immune rabbits. Liquid or dried preparations used in definite quantity (2 units).	Human corpuscles washed or unwashed, but should not contain fibrin ferment. 1 c.c. of approximately 1 per cent.	Fresh or old specimens can be tested. Even a dried serum is eligible for the test. Definite, adjustable quantities. Usually only one drop from a capillary pipette. When inactivated serum is used 4 drops are required (.08 cc.)	Adequate quantity in liquid form or dried on paper with inactivated serum, aqueous or alcoholic extract or pure acetone; insoluble lipoids can be used. With active serum only the pure lipoids must be used, in order to avoid nonspecific reaction.

physician who is in the habit of doing laboratory work, after some training in this phase of hæmolytic work. Perhaps of more real advantage to the profession is the circumstance that blood-serum collected by the physician may be sent to a public laboratory and there examined. The writer's system renders the test so simple and easy that the method may well be placed on the list of regular examinations made by most hospital laboratories. The details of the method, with directions for preparing the reagents for use and the detailed directions for carrying out the test and interpreting the results, I have brought together in another chapter of this book. The matter presented in regard to the different forms of the complement-fixation test is summarized in Table 1, on pages 44 and 45.

VII.

A SYSTEM OF SERUM DIAGNOSIS OF SYPHILIS, RECOMMENDED BY THE AUTHOR.

In the following pages it is my purpose to present as briefly as consistent with the necessary detail the method of making a diagnosis of syphilis by serum reaction as it has been developed in my hands. The presentation will, it is hoped, be of interest and service to two distinct sets of investigators, *viz.*, the practising physician who is so situated that he must make his own clinical laboratory tests or pass them by entirely, and those laboratory workers who are concerned either in making laboratory diagnostic tests for others, or are engaged in supplying laboratory reagents in convenient and stable form for others to use. The presentation is accordingly made in two distinct sections:

A. A presentation of the method of work to be followed by those who have obtained their reagents from others and who must make their own tests.

B. A description of the method of preparing the reagents, standardizing them, preserving them in stable form, and using them in fully equipped laboratories.

The interpretation of results will be the same in both instances and to avoid repetition the description of this subject is confined to Section A.

Section A.

METHOD OF WORK TO BE FOLLOWED BY THOSE WHO HAVE OBTAINED THEIR REAGENTS FROM OTHERS AND WHO MUST MAKE THEIR OWN TESTS.

For making the test, aside from the reagents, the following special apparatus will be needed: A few pipettes of 1 c.c. capacity graduated to 0.1 c.c.; two 10 c.c. pipettes graduated to 0.1 c.c.; a few 1 c.c. pipettes graduated to 0.01 c.c.; a number of small test-tubes, the best dimension being 10 x 1 cm. (two tubes will be required for each test and four tubes for controls in each series of tests; the total number needed will, of course, be dictated by the amount of work to be done); a number of larger test tubes or very small flasks for mixing the blood suspension; a number of larger flasks or bottles as containers of physiological salt solution; a number of pieces of thin glass tubing about ⅜ in. in bore for making capillary pipettes. A few test-tube racks with two parallel rows of holes are necessary.

In handling the preparations and glassware absolute asepsis is not required, but it is well to be reasonably clean, bacteriologically speaking. Physiological salt solution (0.9 per cent) should have been

boiled before use and then cooled. Glassware should be thoroughly rinsed with boiling water and allowed to dry without wiping. Chemical cleanliness is essential. The erythrocyte is a delicate cell which is most easily destroyed or altered by many chemical substances in small amounts. Those which are most apt to be encountered ordinarily are soaps, weak solutions of mineral acids and caustic or carbonated alkalies, and bichloride of mercury. Test-tubes which have been in contact with any of these substances must be thoroughly washed and rinsed in clear running water, finally being boiled in pure water and dried previous to use in the test. They should be heated to 200° C. in a dry air sterilizer before use.

Direct preparation for making the test includes the following procedures:

COLLECTION OF SERUM TO BE TESTED.

Only about 2 c.c. of the patient's blood is needed. The writer has found it very convenient to obtain it by puncturing the dorsal side of the last joint of the middle finger rather close to the root of the nail with a sterile Hagedorn needle. Before puncturing compress the finger tightly by coiling about it a small rubber tube or band in such a manner as to drive the blood towards the extremity of the finger. In small children the lobe of the ear should be punctured for greater convenience. The blood will come out in

drops. Collect it in a glass tube of rather large lumen with a capillary end. Wright's capsules are best suited for this purpose. In order to get enough blood it is usually necessary to relieve and replace the tourniquet a few times. One puncture usually suffices. After sufficient blood is collected seal the capil-

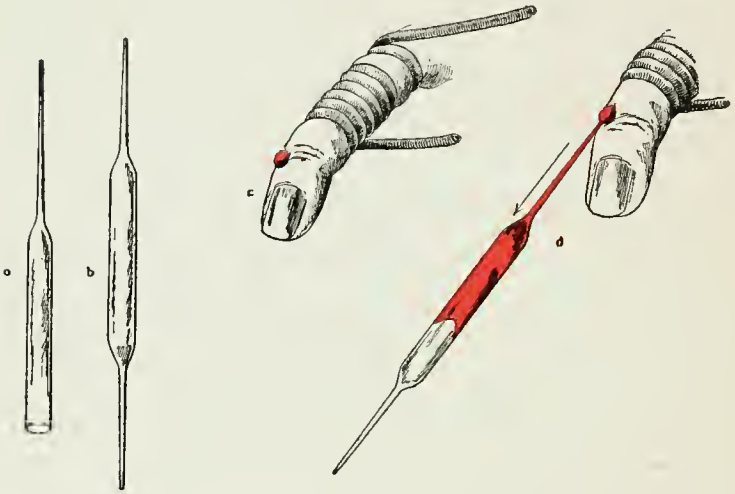


FIG. 7.—*a*, blood-collecting tube; *b*, the same for transportation (both ends can be sealed); *c* and *d* show a simple method of collecting the blood from an adult.

lary end with a flame. During collection utilize both capillary attraction and gravity by holding the tube downward obliquely or perpendicularly. If the blood is to be transported, use a tube with both ends drawn to capillary diameter and seal after blood is collected. Either kind of tube may be made by drawing out ordinary thin glass tubing in the flame of a good-sized alcohol lamp, or, better, a Bunsen burner.

The blood clots and the serum separates in a few hours at room temperature. If the test is not to be made within two or three days the serum should be drawn off with a capillary pipette for storage. If

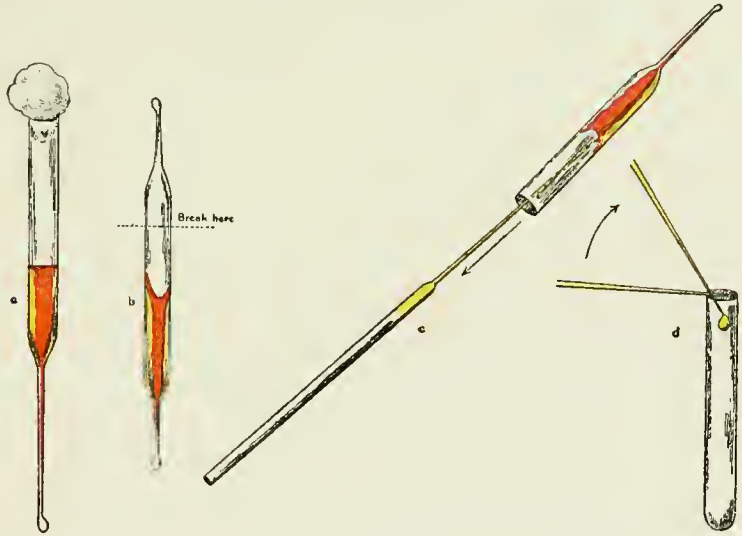


FIG. 8.—*a* and *b* show two blood-collecting tubes with specimens of blood. The clot is retracted from the wall and the space around it is filled with clear serum. *c* and *d* show steps for taking out the serum and placing it in a hæmolytic test tube by means of capillary pipette.

left in contact with the clot it will finally become tinged with hæmoglobin and this will somewhat interfere with the accurate reading of the test subsequently (Figs. 7 and 8).

PREPARATION OF THE CORPUSCLE SUSPENSION.

The suspension can be prepared with the blood of a normal person. It may also be prepared from the blood of the patient being examined. This latter

suspension is, however, to be used with the serum of the same case only. It cannot be utilized for any other case. The advantage in using the native erythrocytes in each case is to eliminate any injurious effect which the serum of certain patients suffering from say carcinoma might occasionally exert upon the corpuscles of a normal person. Besides, if the patient's own corpuscles are used, one can economize in the quantity of the suspension of normal corpuscles necessary when one is working daily with a large number of cases. It is understood that if the corpuscles of the case under examination are unsuitable for use the normal corpuscle suspension must be used for such examination. As it is absolutely necessary to provide a control set in making a diagnosis the corpuscle suspension of normal blood must always be made whether the patient's erythrocytes are utilized or not. I will therefore first describe how to prepare this.

Pour into a large test-tube 8 c.c. of normal salt solution. Draw blood from a normal person in the manner before described. (This operation can easily be done on one's self.) Instead of collecting the blood in a tube allow it to drop into the test-tube of salt solution. The proportion should be one drop for each four c.c. The quantity of the suspension thus prepared is enough for one complete test with some left over in case it is necessary to repeat the test. The suspension must be allowed to stand over

night in a cool place. The next day one finds the cells all gravitated to the bottom. In a laboratory the blood can be washed soon after collection by centrifugalization with an excess of salt solution. Pour off the supernatant fluid carefully and replace it with a fresh lot of salt solution and make a suspension by shaking. This operation removes the serum constituents, and especially the fibrin ferment, which often disturb the test. When one intends to utilize the normal erythrocyte suspension in combination with a patient's serum, the amount of the suspension to be prepared will vary more or less according to the number of specimens to be tested. When one has defibrinated normal blood the suspension may be made shortly before the test is to be done. Removal of the serum constituents by washing is necessary in this case. *A one per cent. suspension of the washed corpuscles should be used.*

In employing the native erythrocytes, in each case the suspension can be made in the same way as in the case of normal blood. But after having accustomed one's eye to the opacity and color of a proper suspension one can prepare suitable suspensions by using the erythrocytes found in the blood tube. If the number of the cells is not sufficient the clot may be gently shaken up to liberate more erythrocytes into the serum. The simplest way is to utilize the patient's own corpuscles. A little common sense and some experience suffice for this. The use of a

too thick or too thin suspension leads to a fallacious result.

With the apparatus as outlined on hand, the serum collected as described, the corpuscle suspension freshly prepared, and with the other reagents secured from a reliable source, the test can be carried out as will be described in the following pages.

TECHNIC OF THE TEST.

To facilitate the carrying out of the test a rack containing two rows of holes for the tubes as shown in the illustration on page 56 should be used. For each test two tubes are required, one in the front row and its control in the rear row. There will also be two pairs of tubes to serve as positive and negative controls.

Put into each of two small test-tubes front and rear one drop (about 0.02 c.c.) of the serum to be tested from a capillary pipette.[1] Add to each tube 0.1 c.c. of 40 per cent. fresh guinea-pig serum made by adding 1 part of complement to 1½ parts of 0.9 per cent. salt solution. In an emergency or where fresh complement cannot be obtained dried slips of paper each containing two units of complement may be substituted. To the front tube add the slip bearing the antigen. Then to both tubes add 1 c.c. of the one

[1] When using inactivated serum put 4 drops (about 0.08 c.c.) into each tube. Use 0.2 c.c. of cerebrospinal fluid not "inactivated."

per cent. suspension of washed human corpuscles (see Preparation of Corpuscle Suspension). Shake the tubes thoroughly from time to time to distribute the reagents evenly throughout the mixture as they dissolve out of the paper.

With every series of tests it is necessary to carry out two sets of controls as already referred to in beginning the description of procedure, and for this purpose four additional tubes will be necessary. To each of the first pair of these, one in the front and one in the rear row, one capillary drop of a syphilitic serum known to give a positive reaction is added. This will serve as a positive control. To the second pair one drop of normal serum known to give a negative reaction should be added, or the tubes may be left empty. This pair of tubes will serve as a negative control. Now put into each tube complement and into the tubes of the front row only antigen, adding finally 1 c.c. of the corpuscle suspension to each tube.

Place the rack holding these pairs of tubes in a water-bath, thermostat, or warm place not over 37° C. The vest pocket is warm enough and convenient in an emergency when none of the above is at hand. Allow an hour from the time the mixture is made for the antibody to combine with the antigen and for complement to be fixed. If a water-bath is used, 30

minutes is a sufficient length of time. If dried paper complement is used this period of incubation should be extended to twice as long as is the case when liquid complement is used. The contents of the tube are as follows:

Rear: Test serum + complm. (2 units) + O + corpuscle susp. (1 c.c.)
Front: Test serum + complm. (2 units) + antigen + corpuscle susp. (1 c.c.)

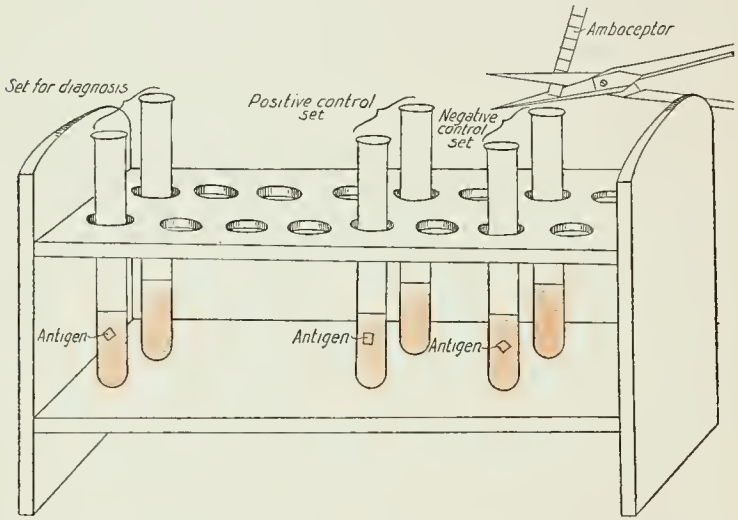


FIG. 9.—The picture shows the appearance of all tubes after the first incubation and just at the moment when the amboceptor slip is about to be added. Thus far there is no visible difference in these tubes and the corpuscles are still intact. In all front tubes there are small square pieces of paper representing the antigen, while none in rear tubes.

First incubation at 37° C. for 1 hour, then add to each tube a slip bearing two units of amboceptor as shown in the illustration, as follows:

Rear: Above + amboceptor (2 units).
Front: Above + amboceptor (2 units).

Allow another two hours in the thermostat or one hour in water-bath. After final incubation the tubes

Front row.	Rear row.
Set for diagnosis. Test with the serum in question	Positive control set. Test with a positive syphilitic serum	Negative control set. Test with a normal serum
a. Unknown serum, 1 drop.*	*a'* Positive syph. serum, 1 drop*	*a''* Normal serum, 1 drop.*
b. Complement, 2 units.	*b.* Complement, 2 units.	*b.* Complement, 2 units.
c. Corpuscle susp., 1 c.c.	*c.* Corpuscle suspension, 1 c.c.	*c.* Corpuscle susp., 1 c.c.
+Antigen.†	+Antigen.†	+Antigen.†
Incubation at 37° C. for 1 hour.		
Addition of antihuman amboceptor, 2 units to all tubes.		
Incubation at 37° C. for 2 hours longer, then at room temperature.		

* When working with inactivated serum 4 drops (0.08 c.c.) should be employed. With cerebrospinal fluid, 0.2 c.c. (not inactivated) is used.

† When using unheated serum pure lipoids prepared by my method (page 71) should be used. With inactivated serum aqueous, alcoholic, or artificial antigen (Sachs and Rondoni) described on pages 88-93 may also be used.

should be kept at room temperature for a few hours before the results are recorded.

The chart (page 57) shows the entire procedure. After the above stages have been carried out, the result of the reaction can be read.

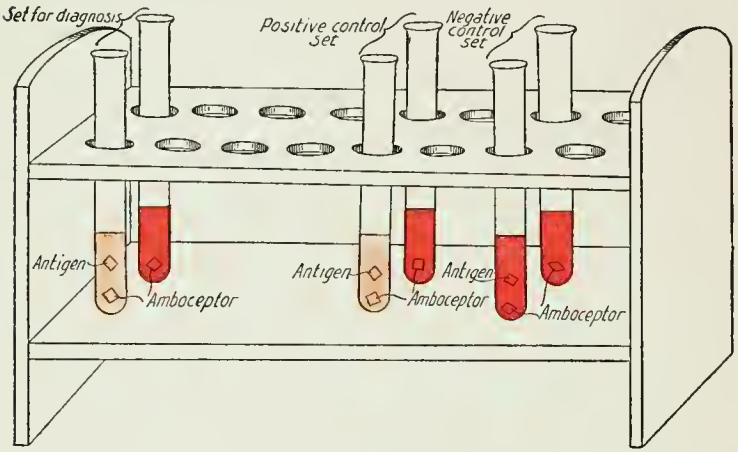


FIG. 10.—This picture shows the appearance of the tubes after completion of hæmolysis, namely, after the second incubation of the same tubes as shown in Fig. 9 with addition of antihuman amboceptor slips. In all front tubes there are two square pieces of paper, one representing antigen and the other amboceptor. In each rear tube there is but one piece, and it represents amboceptor. In negative control set hæmolysis occurred in both tubes. In positive control set hæmolysis took place in the rear tube only and not in the front. In the set for diagnosis the conditions are seen to be identical with the positive control set, hence this serum is found to be syphilitic.

First, it is necessary to make certain that the tests in the control sets have been properly carried out. The pair of tubes containing normal serum (or without any serum) must be completely hæmolysed. These constitute the negative controls and show that the hæmolytic system used is effective (see rear tube) and that the amount of antigen used is not by itself inhibitory of hæmolysis (see front tube).

Next, the front tube of the positive control set, containing a known syphilitic serum, must show total inhibition of hæmolysis, while the rear tube must show complete hæmolysis. Thus we are certain by the rear tube that the syphilitic serum itself does not

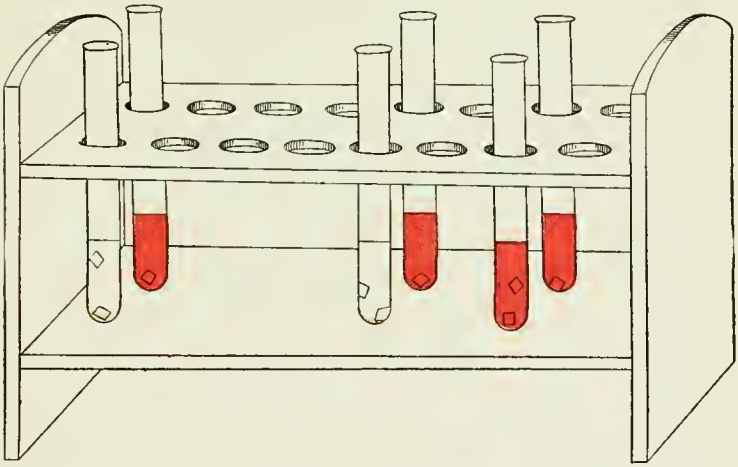


FIG. 11.—This picture shows the appearance of the tubes shown in Fig. 10 after standing for several hours. The absence of hæmolysis in the front tubes of positive control and diagnostic sets is shown by clear supernatant salt solution over the deposited intact corpuscles. The absence of hæmolysis means positive reaction in these instances.

inhibit hæmolysis, while the front tube, in which hæmolysis is inhibited, shows the ability of syphilitic antibody to fix complement in the presence of the antigen employed.

These essentials having been fulfilled, the tubes containing serum for diagnosis can be scrutinized. In these tests hæmolysis must be complete in the rear row, since antigen is not present and the amount of serum used should not be inhibitory. Should hæmolysis be inhibited markedly, showing usually, an anti-

complementary action on the part of the patient's serum, this may be overcome by "inactivation" of the serum for 30 minutes at 56° C. (before commencing), after which it will be necessary to use 4 to 5 drops of the serum in the test. The time of inactivation for this purpose may be shortened to 15 minutes (instead of 30 minutes) and 3 drops of the serum used in this instance. The anticomplementary action can be overcome also by the use of a larger dose of complement. In case inhibition is only slight in amount, the addition of 0.15–0.20 c.c. of a 40 per cent. complement mixture (mentioned already) can be used. The above irregularities are occasionally encountered in the test, especially during the summer months.

Now, the tubes containing the serum for diagnosis and antigen, the front row of tubes, may be examined for final results.

Here any degree of hæmolysis may be encountered, from total inhibition to complete dissolution of corpuscles, depending on the presence or absence of syphilitic antibodies and the number of antibody units. With complete inhibition of hæmolysis, the end reaction is easily interpreted, the corpuscles settling to the bottom of the tube with the clear salt solution above (see the front tube of the pair at extreme left). Complete hæmolysis likewise gives a result easy of interpretation, for the corpuscle mass is

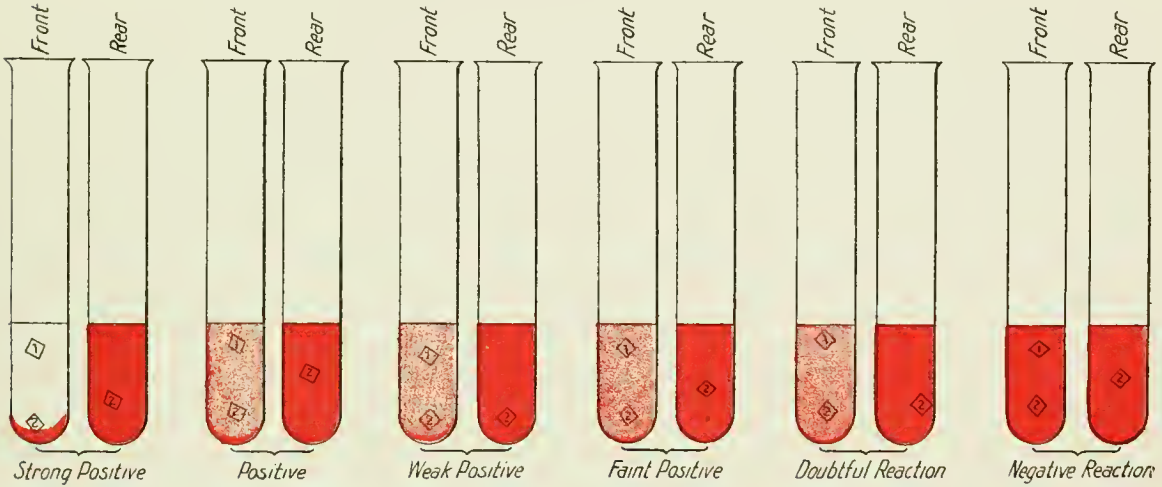


FIG. 12.—Squares marked 1 represent antigen; squares marked 2 represent amboceptor.

entirely dissolved, the hæmoglobin going into solution and coloring the salt solution a deep reddish color (see the front tube of the pair at extreme right).

By taking into consideration the bulk of corpuscles settling to the bottom of the tube and the amount of tinting of the supernatant salt solution, and by comparison with the positive and negative controls, the varying degrees of inhibition of hæmolysis may be ascertained (see Figs. 10, 11, and 12). In interpreting the result complete inhibition of hæmolysis comparable with the positive control is called *strongly positive;* complete hæmolysis, comparable with the negative control, is designated as a *negative reaction.* If 60 to 70 per cent. of the bulk of corpuscles is dissolved the reaction is *doubtful* and should not be taken into consideration for diagnosis. In a known specific case such slight inhibition should be an indication for further treatment as evidence of the continued presence of syphilitic antibodies in the patient's blood. If there is a faint degree of hæmolysis, the main bulk of corpuscles being intact, the reaction should be called *positive.* A more intense hæmolysis, with about 10 to 20 per cent. dissolution of the corpuscle mass, should be called *weakly positive,* while 30 to 40 per cent. hæmolysis is designated as *faintly positive.* Neither the weakly positive nor the faintly positive reaction should be accepted as a definite diagnosis of syphilis without the presence of

strong clinical evidence in favor of such a diagnosis.

In case the reaction should be doubtful, the serum should be re-examined after a period of a week has elapsed, and if necessary several examinations in succession should be made. In all cases it is a wise precaution to take the blood for examination shortly before meal-time.

It may be stated here that all human sera gradually become anticomplementary after several days on standing, some more pronouncedly so than others. This change sets in much more rapidly at a higher temperature, say that of a room, than at a lower temperature, say of a refrigerator.

In order to have a positive control test whenever examining an unknown serum one must always have at hand a syphilitic serum known to give a positive reaction. For this purpose one has to obtain a good specimen, which can be preserved on ice for months. Should such a specimen become too anticomplementary on standing, one can remove this property by heating the serum at 55°–56° C. for about fifteen minutes.

TITRATION OF THE ANTIBODY CONTENT OF SYPHILITIC SERUM.

In the routine examination of patient's serum for the presence of syphilitic antibodies, as above described, one drop from a capillary pipette is used.

This amount of serum is used to determine whether the serum is that of a luetic case or not. When a strong positive reaction is obtained in several specimens we are unable to distinguish the intensity of the reaction without further analysis. In fact, a positive reaction may be got with any syphilitic serum containing many units of syphilitic antibody or that quantity which is just sufficient to deviate complement. A serum may thus contain more than one unit of antibody, and in order to determine the exact strength of each specimen, it should be titrated in the following way.

Mix four drops of the serum with 76 drops of saline solution, making a 1:20 dilution. From this dilution measure into a number of tubes a graded number of drops up to 20, corresponding to one drop of undiluted serum. Thus:

No. of drops.	Equivalent to drops of original serum.
1	1/20
1.5	1/15
2	1/10
3	1/6
4	1/5
5	1/4
7	1/3
10	1/2
20	1

If a given specimen should give complete fixation with two drops, it would be said to contain ten antibody units. By this means the titre of any specimen

can easily be determined. The writer has examined specimens which contained as many as 20 units to the drop. Approximately speaking, one drop corresponds to 0.02 c.c., hence one unit was contained in 0.001 c.c. of such specimens.

The use of the capillary drop is sufficiently accurate for all practical purposes.

Section B.

METHODS OF PREPARING THE REAGENTS AND OF MAKING A COMPLETE FIXATION TEST WITH PREPARATIONS OF UNKNOWN VALUE.

COMPLEMENT.

The views of the author regarding the use of complement dried in paper have been modified within the past few months and now he considers it advisable to use the liquid complement whenever possible. While it is quite possible to prepare the complement on paper its use should be reserved for emergencies—when the fresh complement cannot be obtained.

Guinea-pig's serum is to be used. Large animals are selected and bled by cutting the carotid artery, allowing the blood to flow into a large Petri dish. The dish is then covered and left at room temperature for several hours for the clot to form and the serum to separate. Then the separation of the serum may be

completed in the refrigerator. Within five to ten hours all the serum has separated from the clot and should then be poured into a sterile test-tube and thereafter when not in use kept in the refrigerator. No preservative may be added. After the serum is 48 to 72 hours old the activity of complement is rapidly lost, even at refrigerator temperature, if the serum is kept in a fluid form.

The author does not advise the use of complement dried on paper if it is at all possible to obtain fresh guinea-pig's serum. However, mindful of the fact that this is difficult if not impossible of accomplishment under certain circumstances, as, for example, in out of the way places, army camps, etc., the method of preparing complement paper will be described.

Preparation of Complement Slips.—Squares of thick blotting-paper are put in a sterile flat dish and serum is poured over it until the paper is thoroughly soaked and an excess remains. The saturated paper is then removed to another dish or flat tray and *quickly* dried in a current of air at a temperature not above 10° C., the lowest possible temperature being the most useful. The drying should be accomplished within an hour. According to the thickness of the paper a second impregnation is recommended. After complete desiccation the paper is standardized in the following way: Use a hæmolytic system composed of

human erythrocytes and an antihuman amboceptor. The erythrocyte suspension is to be that used in our test, that is, 1 c.c. of one per cent. suspension of human corpuscles. The amboceptor must have been first standardized with fresh fluid guinea-pig complement. Arrange a series of tubes each containing 1 c.c. of erythrocyte suspension and one unit of amboceptor. Now, selecting a sample of the paper impregnated with complement, cut it into strips of a given dimension in millimetres, preferably 5 mm. wide. Add to the series of tubes bits of paper of increasing length, say 2, 3, 5, 7, 10, 15 mm., etc. Incubate the mixture at 37° C. for two hours. That size of slip in which hæmolysis is just complete will contain one unit of complement. As we desire to use two units of complement in the fixation test, the remaining paper will be measured off into squares having *twice* the dimension of that found for one unit. These may be cut into squares, or, what is perhaps more convenient, marked with pencil so that they may be snipped off as they are needed. These squares or strips must be kept perfectly dry. When they are to be kept in bulk for any length of time it would probably be a wise precaution to keep them in a desiccator over calcium chloride, or in a hermetically sealed tube.

PREPARATION OF AMBOCEPTOR.

Antihuman hæmolytic amboceptor is to be used. This is made by immunizing rabbits against human blood-corpuscles. Select large rabbits and inject increasing amounts of washed[1] human blood-corpuscles five times in succession intraperitoneally, allowing a four- or five-day interval between injections.[2] Nine or ten days after the last injection, bleed the rabbit from the carotid artery with a blood-tube shown in the drawing on page 68.

SCHEDULE FOR IMMUNIZATION.

Injections at four- or five-day intervals. Bleeding nine or ten days after the last injection.

1st injection,	5 c.c. of	the	washed	human	corpuscles.
2d injection,	8 c.c. of	"	"	"	"
3d injection,	12 c.c. of	"	"	"	"
4th injection,	15 c.c. of	"	"	"	"
5th injection,	20 c.c. of	"	"	"	"

After the blood is collected the blood-tube is placed at room temperature for several hours before being transferred to the refrigerator. During this period the clot gradually contracts and separates from the wall of the tube, allowing a clear serum to exude in the space between the clot and the wall of the tube.

[1] The corpuscles must be washed at least three times with a large amount of saline solution. If this is not done the immune serum may contain precipitin for human serum which will interfere with the fixation reaction.

[2] Three or four intravenous injections, 4 c.c., 3 c.c., 4 c.c. and possibly another 4 c.c. with four- or five-day intervals, give also good results. This mode of immunization is, however, less safe for the rabbits.

If the clot remains uncontracted within four or five hours, separate it carefully from the wall by inserting a sterile stiff platinum needle or glass rod, and

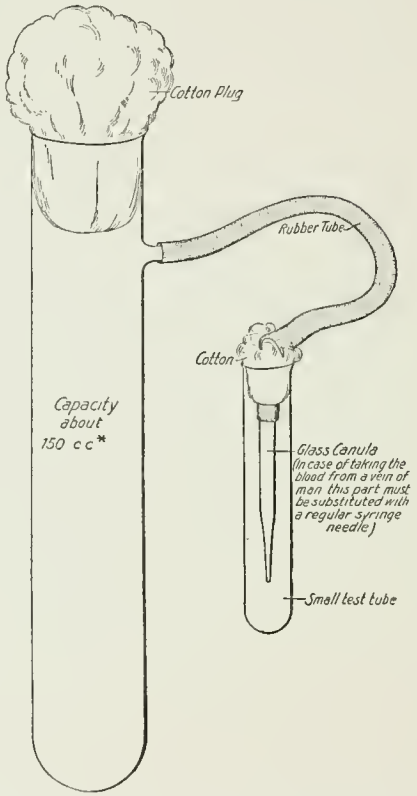


FIG. 13.—Sterilize in steam, but not in hot air. For the purpose of taking the blood from man the size of the tube should be that of ordinary test-tube and should be provided with a good-sized syringe needle instead of a glass canula.

*This can be smaller, although less convenient than a larger one.

allow it to stand at room temperature for several hours again to promote the contraction of the clot. Then place the blood in a refrigerator for twenty-

four hours. Collect the clear serum at the end of twenty-four hours by decantation, and leave the tube for another day to collect serum again. Repeat this for three or four successive days, until no more serum is given out by the clot on further standing. The portions of the serum collected in this manner over several days may be mixed together. When the serum contains a certain amount of corpuscles let it stand for a day or longer in an ice-box to allow the latter to sink to the bottom, and collect the clear serum by gentle decantation, or centrifugalization of the bloody serum may be resorted to.

The amboceptor had best be titrated in fluid form when collected to be sure it is strong enough, before going to the trouble of impregnating paper with it.[1] The principles of the titration were fully discussed in Chapter II. A good preparation will have a value of 1 unit in something less than 0.001 c.c. of serum, that is, 0.001 c.c. of serum or less will cause complete hæmolysis of 1 c.c. of a one per cent. suspension of human erythrocytes when combined with an excess of complement, say 0.02 c.c. of guinea-pig's fresh serum.

Method of Preparing Amboceptor Slips.—When it has been determined that our preparation is

[1] Different rabbits react differently to amboceptor production and it is not seldom to get a weak serum after a long immunization, while a powerful serum may be gotten after four injections.

of the required strength, as will usually be the case if the method of immunization outlined is followed, we proceed to the preparation of the paper. This is a more simple matter in the case of the amboceptor than in the case of complement, for the former is stable. As our slip will only contain about 0.001 c.c. of serum in place of 0.04 c.c., a thinner paper is used. I have found Schleich & Schull's paper No. 597 satisfactory. The paper is cut into squares of about 10 × 10 cm. and soaked with the serum in the same general way as is the complement, but here I avoid too great an excess, seeking only to get all the sheets evenly wet, then absorbing the excess with another sheet of paper. The paper can then be dried at room temperature by placing each square separately upon a clean sheet of non-absorbent (unbleached) muslin. Several hours' drying usually suffices. The sheets when thoroughly dry are cut into convenient width, say 5 mm., and then standardized. Take a series of tubes containing one cubic centimetre of the erythrocyte suspension as described for the titration of complement. Add to each definite amount of complement (0.02 c.c.) which is estimated to be an excess over that really required. Then add to the series measured increasing lengths of the amboceptor strip, *e.g.*, 1 mm., 2 mm., 3 mm., etc., and incubate the series for two hours. The shortest strip which causes

complete hæmolysis in this time contains 1 amboceptor unit. The strips are then marked into sections of *twice* this length and cut off at the time of doing the test. Each section will then contain the two units to be used in the test. These papers should be kept dry and sealed, but the same extreme precautions need not be taken with them as with the complement paper.

PREPARATION OF ANTIGEN.

It is settled to-day that alcoholic extracts of certain tissues contain variable quantities of "antigen" for syphilis. There is more in heart, liver, or kidney than in nervous tissues, so far as has been determined. The liver of a congenitally syphilitic fœtus is one of the tissues richest in these antigenic lipoids. I have found that not only lecithin, but also several other phosphatids, as well as several acetone-soluble fractions of tissue lipoids, can act as antigen. It is not at all improbable that the function of these lipoids is only an intermediation between the real syphilis antigen and antibody present in the serum of a syphilitic patient.

Extract a mashed paste of liver, heart, or kidney of man, ox, guinea-pig, rabbit, or dog with 10 parts of absolute alcohol at 37° C. for several days. Filter through paper and collect the filtrate. The latter is then brought to dryness by evaporation with the aid of an electric fan. The residue is then taken up

with a small quantity of ether and five volumes of acetone added. A precipitate forms, which is allowed to settle to the bottom of the vessel, and the supernatant fluid decanted off. We thus obtain a dark brown sticky mass. This insoluble residue contains antigenic lipoids and its strength must be estimated.

Titration of Antigen.—In order to ascertain whether a given extract is suitable for antigen or not an emulsion is first prepared and tested. If it is found suitable the extract may be impregnated in paper and used in the form of dried strips. I will describe here the general way of determining the antigenic value of a given extract.

Weigh out 0.2 gram of the sticky mass just mentioned and dissolve it in about 5 c.c. of ether, then add gradually 100 c.c. of physiological salt solution to make an emulsion. If flocculent it may be filtered through paper to remove the precipitate. Use this opalescent emulsion for the fixation test, that is, to find out the quantity of the emulsion needed to cause complete inhibition of hæmolysis (fixation of complement in other words) when combined with a good syphilitic serum. It should be noted that any extract-emulsion has a more or less inherent anticomplementary property; it causes fixation of complement without the presence of syphilitic antibody. The extract emulsion is a mixture of a large number of various

lipoids and fats, and it is not to be wondered at that one preparation may contain more antigenic than anticomplementary substances, or *vice versa,* according to the conditions under which a tissue has undergone extraction. Whether the antigenic property of an emulsion is possessed by the same lipoids that are anticomplementary or by different lipoids is still undetermined. It is well to bear this fact in mind in determining the antigenic value of the emulsion. The following will illustrate the method of standardization:

METHOD OF TITRATION.

Front row of tubes.	Back row of tubes.
1. 0.04 c.c. complement.	1. 0.04 c.c. complement.
2. 1 c.c. blood suspension.	2. 1 c.c. blood suspension.
3. 1 drop of positive syphilitic serum.	3. Normal serum, 1 drop.

4. Decreasing doses of the antigen-emulsion to be tested in both series. Incubation at 37° C. for 1 hour, then addition of 2 units of amboceptor. Further incubation for two hours. The results are shown below.

SAMPLE TITRATION.

Amount of antigen emulsion in c.c.	Front row. Inhibition of hæmolysis through the real fixation phenomenon.	Back row. Inhibition of hæmolysis due to the binding property of antigen alone.	
0.4	Complete inhibition.	Slight inhibition.	
0.3	Complete inhibition.	Much inhibition	
0.2	Complete inhibition.	Partial inhibition.	
0.1	Complete inhibition.	None	Complete hæmolysis
0.07	Complete inhibition.	None	
0.05	Complete inhibition.	None	
0.04	Complete inhibition.	None	
0.03	Partial inhibition.	None	
0.02	Complete hæmolysis.	None	
0.01	Complete hæmolysis.	None	
0.007	Complete hæmolysis.	None	
0	Complete hæmolysis.	None	

In the foregoing experiment we find that the antigen alone can prevent hæmolysis when used in a dose of 0.4 c.c. of the emulsion, but is not able to interfere with hæmolysis when a dose below 0.1 c.c. is used. On the other hand, the same antigen can inhibit the hæmolysis completely in a dose as small as 0.03 c.c. if there is present syphilitic serum. The syphilitic serum is of course without any inhibitory action by itself in the quantity here employed. Thus, this inhibition is caused by the co-existence or simultaneous presence of antigen and syphilis-antibody and is of diagnostic value. Now, concerning the antigenic value of the emulsion here employed, we find that any quantity ranging from 0.1 c.c. to 0.03 c.c. is capable of causing complete fixation and may be used for the test. But it is best to choose the maximum dose of emulsion which is without inherent anticomplementary action. In this case let us take 0.1 c.c. as the dose to be used in the test for each tube. There are many samples of organs extracted by alcohol alone without acetone fractionation which do not possess as high antigenic value as this one. Very often the inhibiting dose of emulsion with normal serum is only slightly larger than that required for regular fixation with syphilitic serum. Such preparations are unfit for use as antigen.

The antigen keeps well in the dried state on filter-

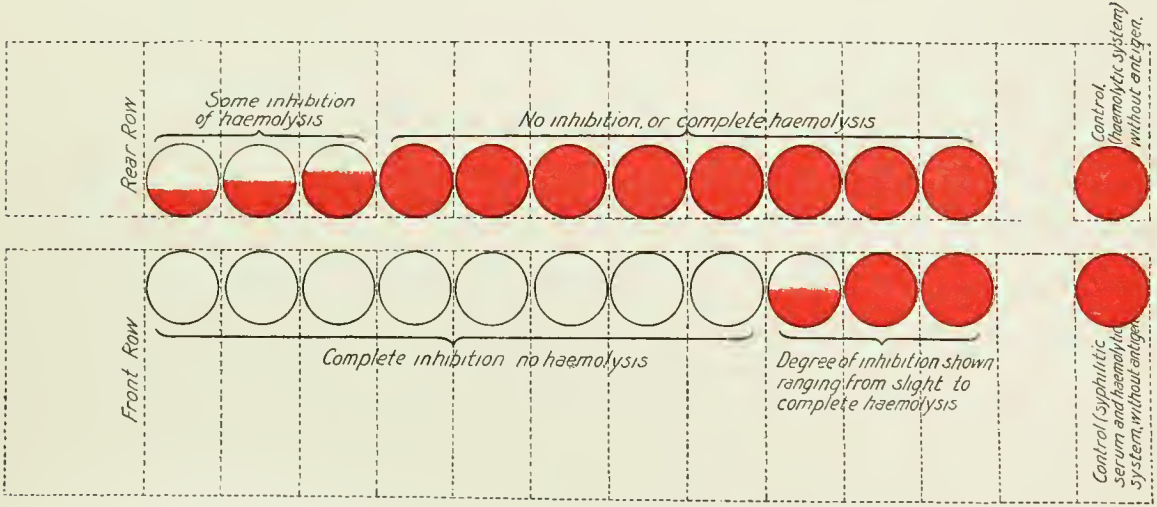


FIG. 14.—The dose of antigen solution is gradually reduced towards the right of the series. The first pair received 0.4 c.c. each, and the pair next to the last, 0.007 c.c. The last pair had no antigen at all. In the front row tubes one drop of syphilitic serum each, but in the rear, one drop of normal serum (compare the protocol). Red means hæmolysis. Blank means no hæmolysis.

paper and is more convenient to use in that form than in liquid form.

The process of impregnating filter paper with antigen is similar to that used for amboceptor. Here we use ethereal or alcoholic solution of the antigenic lipoids for impregnation.

Method of Preparing Antigen Slips.—Weigh out about 0.4 grams of the sticky mass of the extract and dissolve in about 20 c.c. ether. Have ten sheets of filter-paper of the dimensions of 10 × 10 cm. ready, laid one upon the other in a clean glass dish. Pour over these the lipoid solution and saturate the paper evenly. Separate each sheet as quickly as possible and lay flat on a clean sheet of unbleached muslin, as in the case of the amboceptor paper. Evaporation of the solvent usually takes place very quickly and within ten minutes the impregnated paper is ready for use. Before assigning the dimension for each tube in the fixation test the antigen paper should be titrated. This is done in the following manner. Cut the paper into equal width, say 5 mm., and use increasing lengths of this strip for standardization, starting with 1 mm., 2 mm., 3 mm., etc. The principle of standardization of antigen slips is the same as described for the liquid preparation, differing only in using paper instead of liquid. That dimension which does not by itself cause much inherent inhibition of

hæmolysis, but which inhibits hæmolysis completely with syphilitic serum is recommended for use in the test. The strips may be marked in sections, each representing the required dimensions, and put into sealed tubes for preservation.[1]

I may say finally that with every facility for using the liquid preparations I still find it more convenient to use the antigen and amboceptor dried on paper. The complement is more difficult to prepare in this form and I continue to use it in fluid form and so advise its being used wherever possible.

[1] These antigen strips may sometimes become highly anticomplementary and lose, at the same time, the antigenic properties within two or three months. It is necessary, therefore, not to use the strips that become anticomplementary during preservation. It is easy, whenever necessary, to prepare enough strips to last for two or three months from the stock material of the acetone insoluble lipoids, as the latter remains unaltered for at least one year. A liquid emulsion of the antigen may also be employed in the test, but it should be prepared freshly every week or so and kept constantly on ice.

VIII.

ADJUSTABILITY OF THE WRITER'S SYSTEM.

Under ordinary circumstances the relative quantities of the different factors prescribed in my system should give uniform and reliable results; but, as the activity of the complement and the resistance of the red corpuscles sometimes vary according to their source and age, certain irregularities now to be considered sometimes arise. In view of these variables it is desirable that the worker understand how to adjust relatively the quantities of these factors. Indeed, there are no difficulties that can arise which cannot be removed by the proper use of the several reagents.

1. One sometimes meets with instances in which the hæmolysis is complete within 10 to 20 minutes, and in which the positive control tubes with antigen undergo, sooner or later, gradual hæmolysis. Such rapid progress of hæmolysis at first mentioned is a sign of imperfect reaction. If the test is properly made, hæmolysis proceeds gradually, and is complete in the water-bath within half an hour or thereabout. The causes of this accelerated hæmolytic process are either an abnormally weak resistance of the blood-corpuscles, or an exceptionally high activity of the complement employed;

or it may be the result of both these acting together.[1] The corpuscles should never be older than 72 hours, and should be kept constantly on ice, except when being used for the test. They decrease rapidly in resistance after the 72-hour period, and more quickly if kept at room temperature. Sometimes the corpuscles, even if freshly drawn, undergo an abnormally quick hæmolysis, which is to be explained by the fact that in some pathological states—in tuberculosis for example—the corpuscles are subnormal in point of resistance to hæmolytic agents; such corpuscles are, therefore, not suitable for use in this reaction. Having even chosen suitable corpuscles, the hæmolysis may still proceed too rapidly, in which case the complement is likely to be at fault. It happens occasionally that the serum of certain guinea-pigs contains an abnormally active complement. In order to establish this point, and thus to remove this source of error, one has only to make the test with a smaller quantity, say a half dose, of the complement, or, speaking more correctly, a quantity that corresponds nearly to two complement units.

2. There are sometimes encountered instances in which hæmolysis remains incomplete even in the control tubes in which there is no antigen. Here the causes of the imperfect reaction are found either in

[1] Guinea-pig's complement may sometimes remain unfixed and mask the positive reaction. It is always best to use a mixture of the sera from two or more guinea-pigs.

the weakness of the complement, or the amboceptor used, or both. Usually the cause is the weakness of the complement, which, owing to its great lability, is likely to deteriorate. Thus it is a good practice to employ complement that is not older than 48 hours, and that has been kept constantly at refrigerator temperature. When the complement is merely weakened by deterioration, a satisfactory result can be obtained by employing larger amounts of it, but on the whole it is far better to obtain a fresh sample and to discard the deteriorated specimen. The activity of the amboceptor is far less subject to external influences which bring about its deterioration, and it is therefore extremely rare to find that the imperfection in the reaction arises from this source.

In testing several specimens of serum at one time it happens not infrequently that some specimens are slower in completing the hæmolytic reaction than others. The cause of this slowness is not present in the complement or amboceptor, but in the specimens themselves. In such cases the specimens are found to contain anticomplementary substances which react with and reduce the activity of the complement. To remove this source of error, it is necessary to employ with these particular specimens a larger quantity of the complement or *to heat the serum to 56° C. for thirty minutes and use four drops for the test.* The difficulty may be obviated in

some cases by collecting specimens of serum to be tested just before meal-time, because the anticomplementary substance is closely associated with the absorption of the chyle into the circulation soon after the meal.

3. The quality and quantity of the antigen can also be sources of error. If one uses poor antigen, either there will be no positive reaction at all, or weak positive reactions will be entirely overlooked. If, on the other hand, an excessive amount of antigen is employed certain nonspecific weak reactions may become manifest, or a false positive reaction even may be obtained, as the result of the action of anticomplementary substances sometimes contained in preparations of the antigen. These sources of error can be entirely excluded by choosing an antigen that has been carefully prepared and standardized by an experienced serologist, which is, indeed, one of the advantages which the employment of my system offers.

Apart from these suggestions, which are essential in order to obtain reliable results with the present system, a few words may be added concerning the making of the reactions on a larger scale. In other words, if it is desired to make the test with larger quantities, one has simply to multiply the quantity of each factor employed. Thus one may use 0.1 c.c. of the complement, 0.05 c.c. of the patient's serum, 1 c.c. of a 5 per cent. suspension of the washed human

corpuscles, and 2 units of the amboceptor (titrated with the above complement and corpuscle-suspension unit), in a total volume of 5 c.c. of physiological salt solution. This increase in the relative quantities of each constituent offers one advantage and one disadvantage. The advantage is that the intensity of the reaction can be more minutely measured through the liberation of the hæmoglobin, as the number of red corpuscles is, of course, much larger. The disadvantage arises from the unnecessary waste of material. For the worker in a regularly equipped biological laboratory this waste may make but little difference, but for those who intend to do the test in a private laboratory the exercise of economy is highly desirable.

When the serum to be tested has previously been inactivated by being heated to 56° C., the amount of serum used must be from four to five times greater than that prescribed for the fresh or unheated serum, since one effect of the inactivation is to reduce the content of the antibody to about one-fourth or one-fifth of the original strength.

In the following chapter the writer will point out the effect of inactivation upon the antibody content of serum. This has bearing not only on what has been said above, but with equal if not greater directness upon the original Wassermann and other systems requiring inactivation of the patient's serum.

IX.

INACTIVATION OF THE SERUM IN RELATION TO THE SYPHILIS REACTION.

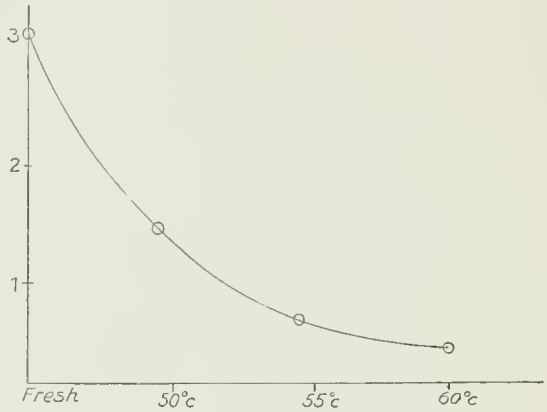
It will be recalled that the serum of patients to be tested is employed either in the fresh state or after inactivation at 56°C. According to the method used in the systems of Wassermann, Detre, Bauer, Boas, and Browning, the serum is previously heated to 56° C. for half an hour, in order to destroy all the native complement present in it. On the other hand, in the systems of Hecht, Stern, and Tschernogubow the serum is employed in the fresh state, since in these systems the native complement is utilized. Unlike these two sets of systems, the one which I offer enables one to use either fresh, or old, or inactivated serum, the only difference being that when the serum has been inactivated a somewhat larger quantity of it must be employed.

We will now consider the reasons why one set of workers employ for the reaction the inactivated, and another the fresh serum. Wassermann, Bauer, and others inactivate the serum simply to destroy the native complement, which varies in different specimens of serum, and in order to substitute this un-

known content by a uniform amount of guinea-pig complement of known activity. Hecht and Stern, however, found that when the test is made with fresh serum, so as to employ the native complement, the reaction is more sensitive than when the inactivated serum is used. On what does this greater delicacy of reaction of the fresh serum depend? Since this point has not been touched upon, I have made a careful study of it, as a result of which I am prepared to offer an explanation of the difference existing between fresh and inactivated serums.

The first question which I asked myself was: Is the so-called syphilitic antibody affected in the process of inactivation? It had previously been found that this antibody is completely destroyed at temperatures between 72° and 80° C. in about twenty minutes. I found that the spinal fluid loses its antibody when heated to from 75° C. to 80° C. for twenty minutes, as had been previously found by Marie and Levaditi. The syphilitic serum I observed to have become inactive at 72° C., but the coagulation of the protein interfered in a high degree with exact observation. We know, therefore, with fair accuracy the limit of temperature at which the total destruction of the antibody is established, but we know almost nothing of the rate of destruction which takes place at lower temperatures. Sachs states that the antibody content

of syphilitic serum is considerably reduced by temperatures of 60° C. I have found, on subjecting a specimen of serum from a case of untreated secondary syphilis to temperatures of 45°, 50°, 55°, and 60° C. for twenty minutes and then determining, by fixation tests, the amount of the antibody available, that the



CURVE 1.—Heating of a syphilitic serum to different temperatures for 20 minutes (in a water-bath).

syphilitic antibody is greatly reduced even at 45°. At 50° C. it is reduced to about one-half, at 55° C. to about one-fourth, etc., as is shown in Curve 1. I next studied the rate of destruction of the antibody at the temperature of 55° C., at five, ten, twenty, thirty, and sixty minute periods. The results were rather unexpected, since the rate of destruction is greatest during the first five minutes, during which time the antibody strength is reduced about one-third

of the original. After thirty minutes it has been reduced one-fourth to one-fifth, and at the end of one hour to about one-tenth of the original, as can be seen by reference to Curve 2. In studying the serum from a case of leprosy, I found that the diminution of the antibody strength went on in precisely

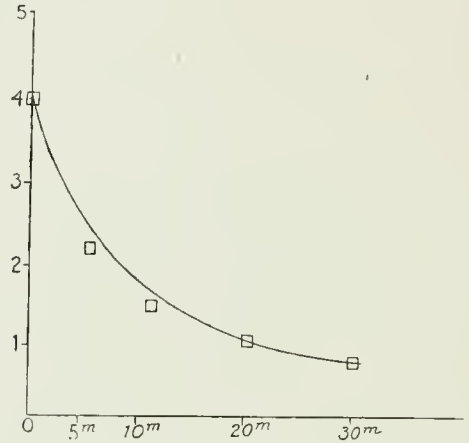
CURVE 2.— Heating of three different samples of syphilitic sera to 55° C. for varying lengths of time (in a water-bath).

the same way as in the specimens obtained from syphilis (Curve 3).

It has been stated by certain investigators that the fresh serum of nonsyphilitic cases—as for example, cases of carcinoma—may give a positive reaction, and that this reaction disappears when such a serum has been previously heated to 56° C. for thirty minutes, and that therefore the two groups of positive reaction, specific and nonspecific, can be distinguished

by the employment of inactivated serum. I have not been able to find any very accurate studies of this topic, which on the whole is so very important.

My recent studies on the Bordet-Gengou fixation phenomena in general revealed an unexpected fact, namely, that in the majority of active human sera



CURVE 3.—Heating of a serum from a case of leprosy to 55° C.

irrespective of sources there exists a constituent which fixes complement when mixed with certain proteins such as nucleoproteins, pepton, albumoses, and many other autolytic decomposition products of proteins. This peculiar phenomenon is of course nonspecific, but difficult to differentiate from the real specific Bordet-Gengou as well as Wassermann reactions. Fortunately this false reaction disappears when the serum is previously heated to 55° C. for thirty minutes.

Thus, no active human serum should be employed for fixation test when these proteins can not be excluded from the extracts serving as antigens. Considering the Wassermann reaction in the light of this revelation it becomes at once evident that none but the inactivated sera should be used for the test when aqueous or even alcoholic extracts of macerated organs of fœtus are employed as antigens, because of the presence of these proteins in the extracts. This explains clearly why certain investigators obtained positive reactions in some nonsyphilitic cases by using active sera. On the other hand, I have found that active human serum does not give such nonspecific reaction when mixed with aceton-insoluble lipoids. The results obtained by using active human serum, and the lipoids just referred to are comparable to those obtained by inactivated serum and thirty other suitable syphilitic antigens and are perfectly specific.[1]

[1] It is an erroneous conception that my system uses only active serum. On the contrary, it can use an old or inactivated serum as well. Whether the active, old, or inactivated serum is used is only a matter of personal choice, provided that the rules I prescribed for active and inactive sera are observed.

X.

TECHNIC OF THE WASSERMANN SYSTEM.

In performing the test by the original Wassermann system five different factors are required, viz.: antigen, patient's serum, complement, amboceptor, and blood-corpuscles. The source and mode of preparation of these factors will be given in detail below.

PREPARATION OF ANTIGEN.

A. AQUEOUS EXTRACTS.

1. *Wassermann's Method.*—The liver or spleen of a congenitally syphilitic fœtus is preferred. Take the organ and cut it up into very small pieces with a pair of scissors and mix the tissue with four parts of physiological salt solution to which phenol in the proportion of 0.5 per cent. is added. For example:

360.0 c.c. salt solution (0.85 per cent.)
100.0 grams liver
40.0 c.c. phenol (5 per cent.)

This mixture is thoroughly shaken in a dark bottle for twenty-four hours by means of a shaking machine. The tissue pieces are separated by centrifugalization and the brownish, opalescent supernatant fluid is used for antigen. It should be preserved in a rubber-stoppered dark flask in the refrigerator. Upon

standing a precipitate falls to the bottom of the container. This precipitate should not be used. As much of the clear supernatant fluid as is necessary for the day's work should be poured off, and the remainder put back on ice immediately.

As to the stability of this extract, there is no agreement among investigators, whose experiences differ widely upon this question. Wassermann, Neisser, Bruck and Schucht found that it is very unstable, soon becoming too anticomplementary for use. Citron once prepared a watery solution of antigen which he divided into three portions. He kept one part for his own use and the other two he sent to other laboratories. One of these reported to him after the lapse of a week that the antigen had become inactive; the other sent a similar report after four weeks. The portion which he kept was unaltered three months after its preparation. So that it would seem the stability of the antigen depends greatly upon the mode of its preservation. My own experience shows that the liver of every congenitally syphilitic fœtus does not always yield a good antigen and that when once prepared in the above manner it may deteriorate within a few weeks.

For the extract used in the control tests a normal organ should be similarly prepared.

2. *Marie and Levaditi's Method.*—Mash the liver of a congenitally syphilitic fœtus and dry in a vacuum

and then pulverize it. The powder is suspended in four parts of physiological salt solution and the mixture centrifugalized after twenty-four hours extraction. The clear supernatant fluid is used.

3. *Morgenroth and Stertz's Method.*—Preserve the organ (syphilitic liver) in frozen state (*in Frigo*), and cut off a small piece each time for use in the test. Mash this bit of tissue with sea-sand and extract it with four parts of physiological salt solution. Filter through paper, and use the filtrate.

The most important point concerning antigen is to employ the proper quantity in the test. It has been made a general rule that that dose of antigen must be selected which does not bind complement even when the antigen is used in double quantity. The usual aqueous preparation may be used in 0.1 c.c. or 0.2 c.c. doses.

B. ALCOHOLIC EXTRACTS.

1. *Porges and Meier's Method.*—Cut up a normal or syphilitic liver into small pieces, extract with five volumes of absolute alcohol for twenty-four hours, and filter through coarse filter-paper. The filtrate is evaporated in a vacuum at a temperature below 40° C. The sticky mass resulting is then used to prepare a 1 per cent. suspension in physiological salt solution with the addition of 0.5 per cent. of phenol. This emulsion is well shaken and filtered through fine paper. The minimal dose which shows inhibition of hæmolysis is

determined by titration, and half of this amount is used for the test. With a strong syphilitic serum 0.025 c.c. may give a complete reaction, but 0.2 or 0.3 c.c. is usually necessary for the test. The authors found a preparation of lecithin (Kahlbaum) to be equivalent in antigenic property to the alcoholic extract, but later investigators have found such a preparation unreliable as an antigen.

2. *Landsteiner, Müller, and Pötzl's Method.*—Extract mashed guinea-pig's heart or liver with alcohol for about ten to twelve hours at 60° C., in the ratio of one gram of tissue to 50 c.c. of 95 per cent. alcohol. Filter through paper and preserve the filtrate at room temperature. Use two drops of the solution for the test.

3. *Michaelis and Lesser's Method.*—Shake minced normal or syphilitic liver with ten volumes of absolute alcohol for ten to twelve hours. Use glass beads to facilitate thorough extraction. After twenty-four hours the clear supernatant portion is poured or pipetted off and used as antigen. Every time the test is to be made one part of this extract is mixed with four parts of physiological salt solution and 1 c.c. of this emulsion is used. The emulsion becomes milky and tends to form a precipitate on standing and should be thoroughly shaken before using. Recently Michaelis has advocated the use of an alcoholic extract of normal human heart.

4. *The Writer's Method.*—Extract minced tissue of syphilitic or normal liver or kidney (beef kidneys can be used) with ten volumes of 95 per cent. alcohol for about six or seven days at 37° C. Filter through paper and evaporate the filtrate by means of a fan at a temperature below 40° C. The resinous residue which results should be extracted with ether, and this ethereal solution then allowed to evaporate to dryness in the air. Take up the residue of this ethereal extract with a small quantity of ether and fractionate with five volumes of acetone. Separate the sticky precipitate by pouring off the supernatant acetone carefully, allow the remainder of the acetone to evaporate, and preserve the resinous mass in an air-tight jar. For the test make a 0.2 per cent. solution in physiological salt solution by first dissolving the resinous mass in a small quantity of ether and then mixing it with the salt solution. Each preparation should be tested before using to determine its reliability and dosage.[1] Usually 0.1 c.c. to 0.2 c.c. is suitable. Kept on ice the emulsion is fairly stable.

C. ARTIFICIAL ANTIGEN.

Sachs and Rondoni advise the following as a suitable antigen for use in the Wassermann reaction:

[1] The aqueous as well as the alcoholic extracts prepared by any method should be similarly tested before use.

	Mixture A.	Mixture B.
Sodium oleate (Kahlbaum)	2.5	1.0
Lecithin (Ovo—Merck)	2.5	1.0
Oleic acid (Kahlbaum)	0.75	1.5
Distilled water	12.5	5.0
Alcohol ad.	1000.0	ad 1000.0

The authors advise the use of either of these formulæ in a dilution of 1 part of the above to five of physiological salt solution, the reagent being thoroughly mixed with the salt solution. No precipitate should be formed. The test doses advised for each serum to be examined are: 0.15, 0.25, 0.4 c.c.

Schürmann's Method.—This investigator recently published this formula as an antigen: Lecithin, 0.30 gram in 50 c.c. absolute alcohol; sodium glycerophosphate, 0.3 gram in 5 c.c. physiological salt solution. Thirty c.c. of the above are mixed with 5 c.c. lactic acid and 10 c.c. of ammonium vanadinate (1 per cent.).

PATIENT'S SERUM.

To get serum for the Wassermann test blood is drawn from a vein and the serum which separates after clotting is inactivated at 56° C. for half an hour, preferably within twenty-four hours after its withdrawal from the patient. Cerebrospinal fluid should be used without inactivation. The test doses are 0.1 c.c. and 0.2 c.c.

COMPLEMENT.

One cubic centimetre of guinea-pig's serum in 1:10 dilution is used. The serum should not be older

than forty-eight hours, and it should be carefully preserved in the ice chamber when not in use.

AMBOCEPTOR.

The amboceptor for the Wassermann test is produced by immunizing rabbits against sheep-corpuscles. The writer has been very successful in obtaining an amboceptor of high titre by using successive injections of washed corpuscles in doses of two, four, eight, and twelve cubic centimetres at intervals of four or five days, and bleeding the animal nine or ten days after the last injection. The corpuscles should be centrifugalized at least twice with a large quantity of physiological salt solution and the original bulk of the defibrinated blood, which had been marked before centrifugalization, restored by the addition of salt solution. The injections should be made intraperitoneally.

One unit of amboceptor should be determined by titrating against 1 c.c. of a 5 per cent. suspension of washed sheep-corpuscles, using 0.05 c.c. of 1:10 dilution guinea-pig complement. Two units are used in the test.

CORPUSCLE SUSPENSION.

One cubic centimetre of a 5 per cent. suspension of washed sheep's corpuscles is used. The blood should be fresh, not older than three days, and should be kept on ice when not in use.

METHOD OF APPLYING THE TEST.

In applying the test according to the original Wassermann method with a watery antigenic extract, the investigator should use as many test-tubes as are indicated in Table 2.

Put the required amounts of serum, complement, and antigen into the respective tubes, and bring the total quantity of the mixture up to 3 c.c. by the addition of salt solution. Mix the contents of the tubes well and incubate in the thermostat for one hour at 37° C. At the end of this period add to every tube amboceptor and corpuscle suspension in the quantity prescribed above, mix well, and incubate again for two hours. Then remove the tubes to an ice-chest for twenty hours, when the test is complete and the results are ready for reading.

If the test has been properly carried out, there will be complete hæmolysis in every control tube excepting in the tube containing syphilitic serum and syphilitic antigen (positive control, *vide* Table 2). If the control tubes are correct, the tubes containing the sera to be examined can be read for the final result. In this series all the tubes containing other than a syphilitic organ extract should be completely hæmolysed.

In the tubes containing the unknown serum and a syphilitic organ extract, there may or may not be hæmolysis according as the serum contains syphilitic

TABLE 2.—*The Wassermann system (Citron), when aqueous organ-extracts are used as antigen.*

	Syphilitic liver, aqueous extract	Normal liver, aqueous extract	Guinea-pig's complement 1:10 dil.		Five per cent. suspension of washed sheep's corpuscles	Immune anti-sheep amboceptor (litre 1:2000) 1:1000 dil.		Results should be
Patient's serum (56° C.)								
0.2 c.c.	0.2 c.c.	.....	1 c.c.		1 c.c.	1 c.c.		The degree of hæmolysis in these 2 tubes determines the nature of this serum.
0.1 c.c.	0.1 c.c.	.....	1 c.c.		1 c.c.	1 c.c.		
0.2 c.c.	.....	0.2 c.c.	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.1 c.c.	.....	0.1 c.c.	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.4 c.c.	.....	.....	1 c.c		1 c.c.	1 c.c.		Complete hæmolysis.
0.6 c.c.	.....	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
Positive syphil. serum (56° C.)								
0.2 c.c.	0.2 c.c.	.....	1 c.c.		1 c.c.	1 c.c.		No hæmolysis.
0.1 c.c.	0.1 c.c.	.....	1 c.c.		1 c.c.	1 c.c.		No hæmolysis.
0.2 c.c.	.....	0.2 c.c.	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.1 c.c.	.....	0.1 c.c.	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.4 c.c.	.....	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.6 c.c.	.....	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
Normal serum (56° C.)								
0.2 c.c.	0.2 c.c.	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.1 c.c.	0.1 c.c.	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.2 c.c.	.....	0.2 c.c.	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.1 c.c.	.....	0.1 c.c.	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.4 c.c.	.....	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
0.6 c.c.	.....	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
No serum	0.4 c.c.	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
No serum	0.6 c.c.	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
No serum	.....	0.4 c.c.	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
No serum	.....	0.6 c.c.	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
No serum	.....	.....	1 c.c.		1 c.c.	1 c.c.		Complete hæmolysis.
No serum	.....	.....	1 c.c.		1 c.c.	.....		No hæmolysis.
No serum	.....	.....	.....		1 c.c.	1 c.c.		No hæmolysis.

The volume of each tube is now brought up to 3 c.c. with salt solution. Incubation at 37° C. for 1 hour.

The volume of each tube is now 5 c.c. Mix the contents of tubes thoroughly and incubate at 37° C. for 2 hours. Remove tubes then to refrigerator over night and read the reactions after this period.

antibodies or not. In the former event there will be inhibition of hæmolysis, either total or partial; in the latter, the tubes should be completely hæmolysed.

The degree of inhibition of hæmolysis varies according to the amount of syphilitic antibody present; if this is large in amount, or in other words, if complete inhibition occurs in the tube containing 0.1 c.c. serum and 0.1 c.c. antigen, the result can be graphically represented, according to Citron, thus: ++++. If inhibition of hæmolysis is incomplete in the tube containing 0.1 c.c. of serum but complete in that containing 0.2 c.c., the result is expressed thus: +++. These reactions are usually called strongly positive. If the tube containing 0.1 c.c. serum is completely hæmolysed while that containing 0.2 c.c. shows complete inhibition, the result is expressed thus: ++. Incomplete inhibition in the tube containing 0.2 c.c. is expressed thus: +. The last two reactions are called weakly positive. When inhibition in the tube containing 0.2 c.c. is doubtful the result is expressed thus: ±.

THE WRITER'S METHOD[1] OF PERFORMING THE TEST WITH THE WASSERMANN SYSTEM.

The regular Wassermann method can be greatly simplified by the use of alcohol-soluble tissue constituents as antigen. Other investigators, as well as

[1] This should not be confused with the antihuman system already described in Chapter VII.

the writer, have found that there is no essential difference between the antigen extract thus prepared and the aqueous extract, while the former in the hands of the writer has proved much more stable and does away with the necessity of using the normal organ-extract controls, which render the original Wassermann method so complicated and cumbersome. An alcoholic extract of a syphilitic organ is usually rich in antigenic properties, while such an extract of a nonsyphilitic organ is less certain in regard to the possession of this property. However, it has been pointed out in the chapter on the Preparation of Antigens that the acetone-insoluble fraction of the alcohol extract of *any* liver or kidney, whether syphilitic or not, is usually rich in this antigenic property, and should be preferred for use in the test.

In Table 3 (see page 99) the exact method of performing the test is given. It will be noted that while the total bulk after all the reagents have been added is smaller than in the original Wassermann method, the relative proportion of the various factors is the same. A uniform dosage of antigen is used, which, in the experience of the writer, has been sufficent to show the varying intensity of the reaction.

TABLE 8.—*The Wassermann system (Noguchi), when alcohol-soluble constituents of organs are used as antigen.*

	0.2 per cent. saline emulsion of the acetone-insoluble fraction of alcoholic extract of organs	Guinea-pig's complement, undiluted		Ten per cent. suspension of the washed sheep's corpuscles	Immune antisheep amboceptor, litre (1 : 2000), 1 : 1000 dil.		Results should be
Patient's serum (56° C.)							
0.2 c.c.	0.1 c.c.	0.1 c.c.	Mix the contents well and incubate at 37° C. for 1 hour (30 minutes in a water thermostat).	0.5 c.c.	1 c.c.	The total volume is 3 c.c. each. Mix the contents well and incubate at 37° C. for 2 hours (1 hour in a water thermostat). Reading of the results after 4 to 6 hours at room temperature.	These two tubes show variable reactions according to the nature of the serum.
0.1 c.c.	0.1 c.c.	0.1 c.c.		0.5 c.c.	1 c.c.		
0.2 c.c.	.....	0.1 c.c.		0.5 c.c.	1 c.c.		Complete hæmolysis.
0.1 c.c.	.....	0.1 c.c.		0.5 c.c.	1 c.c.		Complete hæmolysis.
Positive syphil. serum (56° C.)							
0.1 c.c.	0.1 c.c.	0.1 c.c.		0.5 c.c.	1 c.c.		No hæmolysis (positive reaction).
0.1 c.c.	.....	0.1 c.c.		0.5 c.c.	1 c.c.		Complete hæmolysis.
Normal serum (56° C.)							
0.2 c.c.	0.1 c.c.	0.1 c.c.		0.5 c.c.	1 c.c.		Complete hæmolysis (negative reaction).
0.2 c.c.	.....	0.1 c.c.		0.5 c.c.	1 c.c.		Complete hæmolysis.
.....	0.2 c.c.	0.1 c.c.		0.5 c.c.	1 c.c.		Complete hæmolysis (should be no inhibition).
.....	0.1 c.c.	0.1 c.c.		0.5 c.c.	1 c.c.		Complete hæmolysis (should be no inhibition).
.....	.....	0.1 c.c.		0.5 c.c.	1 c.c.		Complete hæmolysis.

XI.

DIAGNOSTIC VALUE OF THE WASSERMANN REACTION.

The phenomenon of complement-fixation in syphilis is a type of reaction distinct in itself and differing widely from all other known examples of complement fixation. The principal difference between the two types of phenomena arises from the nonspecific nature of the substances that functionate as antigen in the Wassermann reaction. We have already seen (see page 23) that the complement is absorbed or fixed only when brought in contact with combinations of specific antigens and antibodies. In general it may be said that the specificity of these antigens and antibodies can be compared in a way to the relation which exists between locks and keys, and it can be stated that they do not interact with one another unless they are in exact correspondence. On the other hand the phenomenon of Wassermann is produced by what appears to be a specific antibody and certain nonspecific antigenic substances. Therefore in this case the law of specificity does not operate in the same strict sense as in other known examples of the Bordet-Gengou phenomenon.

I have been able to show that several phosphorized and non-phosphorized lipoids and a few salts can act as syphilitic antigens, and that there is no necessary relation between them and a syphilitic infection. When these substances are brought into combination with the blood-serum or cerebrospinal fluid of syphilitic patients, they alter the fluids in such a manner as to render them able to fix the complement which is introduced into the mixture. It is this peculiarity of the serum of syphilitics upon which the serum diagnosis of syphilis is based. This interesting property of syphilitic serum is produced, it is believed, by certain substances existing in the serum, now generally designated as syphilitic antibody, although actually we are still entirely ignorant of their real nature. There is, however, little doubt that they are reaction products of syphilitic infection, and that they appear constantly in the serum of persons infected at certain stages of the disease.

For the purpose of estimating the value of the complement-fixation test as a clinical method for the diagnosis of syphilitic and parasyphilitic conditions, the writer presents a tabulation of the results of investigators. (See pages 102 and 103.)

In primary syphilis the results vary much, ranging from 98 per cent. (Detre) to 38 per cent. (Hoehne). This difference may be accounted for,

TABLE 4A.—*Blood-serum.*

	Primary syphilis		Secondary syphilis manifest		Tertiary syphilis manifest		Early latent syphilis		Late latent syphilis	
	No. of cases	Per cent. +	No. of cases	Per cent. +	No. of cases	Per cent. +	No. of cases	Per cent. +	No. of cases	Per cent. +
Wassermann, Neisser, Bruck and Schucht	25	91	101	73.3	37	78.4	41	85.4	53	88.7
Citron and Blaschko	64	90	56	98	23	91	67	80	51	57
Bruck and Stern	27	48.2	163	79.1	47	57.4	50	20	79	20
Bruhns and Halberstädter	9	88.9	50	98	16	100	39	43.3	82	28
Ledermann	19	52.6	56	100	27	92	41	75.6	19	36.8
Ledermann	46	61.2	110	98.1	78	96.2	115	83.8	78	53.8
Lesser	56	69	204	91	131	90	118	67	425	46
Noguchi**	33	66.6	120	86.6*	91	72.5	81	48.1	74	44.7
Hoehne	44	38.6	329	79.1	33	63.6	387†	31.3		
Boas	50	60	395	100	63	97.	294†	47		
Detre and Brezovsky	43	98	21	81	35	73				
	416	69.8	1605	89.4	581	78.1	1233	51	861	47

* When treated cases are excluded 98 per cent. positive results were obtained.
† No distinction was made between the late and early latent cases.
** Not including 1800 cases examined by the author's new system.

TABLE 4B.—*Blood-serum.*

Investigators	Hereditary syphilis		Cerebrospinal syphilis		General paralysis		Tabes	
	No. of cases	Per cent. +	No. of cases	Per cent. +	No. of cases	Per cent. +	No. of cases	Per cent. +
Boas and Thomsen	32	87.5						
Bauer	22	100						
Halberstädter, Müller, and Reiche	27	92						
Ledermann	16	100	26	88.5	23	87	68	76.4
Hoehne	24	87.5	12	16.7	30	80	45	60
Noguchi*	4	100	2	50	..	....	22	40.9
Boas	..	....	..	....	42	100	20	80
Nonne	..	....	?	(20)	?	(90)	?	(90)
Lesser	..	....	..	....	62	100	61	56
Frenkel-Heiden	..	....	7	27	14	78.5		
Plaut	..	....	4	25	180	100		
Citron and Blaschko	..	....	10	60				
Stertz	..	....	3	66	45	95.5		
Marie, Levaditi, and Yamanouchi	..	....	..	....	30	59		
Raviart, Breton, Petit	..	....	..	....	72	93		
	125	94.5	64	47.6	498	88.1	216	62.66

*Not including 362 cases of tabes, 192 cases of general paralysis, 48 cases of hereditary lues, 11 cases of cerebrospinal lues examined by the author's antihuman system. See tables 5, 6 a, b, c, 7, 13, 14, 15, 16.

aside from technical considerations, by the state of the infection at the time of examination. Early cases of chancre frequently give a negative reaction. However, in this stage of syphilis the test is not usually necessary excepting in those cases in which a differentiation between chancroidal and mixed infection is desirable, and in cases of suspected intra-urethral chancre.

In secondary syphilis the highest figures are those of Boas and of Ledermann; the former got 100 per cent. of positive reactions in 395 cases, the latter a similar result in 56 cases. The lowest figures are recorded by Hoehne, who got 79.1 per cent. of positive reactions in 329 cases, and by Bruck and Stern, who examined 163 cases with similar results. The variations in these figures cannot well be accounted for unless an analysis of the stage of the disease and the treatment received by the patient at the time of doing the test are taken into consideration. The reaction in this stage of syphilis is fairly constant and a reliable index of the presence of syphilitic antibodies in the patient's serum.

In tertiary lues the figures vary from 57.4 per cent. (Bruck and Stern in 47 cases) to 100 per cent. (Bruhns and Halberstädter in 16 cases). Here again the same uncertainty as to treatment, which may so strongly affect the reaction, applies as pointed out above.

In early latent cases the figures vary from 20 per cent. (Bruck and Stern in 50 cases) to 85 per cent. (Wassermann, Neisser, *et al.* in 41 cases.) By early latent cases is meant those late secondary cases without symptoms. In late latent cases, or those following the manifest tertiary stage, without symptoms, the figures show about the same results. The technic of the various investigators and the reagents used by them must be taken into account in accepting the results of their work.

General paralysis shows fairly constant positive reactions, ranging from 80 per cent. to 100 per cent. of cases examined. Tabes gives a somewhat lower percentage, from 40 per cent. to 80 per cent. In hereditary syphilis the figures are fairly constantly high, the lowest being 87.5 per cent. In cerebrospinal lues the results vary from 16 per cent. in 12 cases reported by Hoehne to 88.5 per cent. in 26 cases reported by Ledermann.

The writer interpolates (Table 5) the results obtained by him with his own system in 1082 cases of syphilis in its various manifestations and stages, and a comparative study of syphilis and parasyphilitic conditions in 244 cases analysed by the above system and the regular Wassermann system (Table 6A). In Tables 6B and C, comparative studies of the Wassermann and Noguchi systems made by Fox on 210 cases and by Kaplan on 1286 cases are presented.

The results of the analysis of the cerebrospinal fluid in general paralysis vary from 73 per cent. (Marie and Levaditi, and Noguchi and Moore) to

TABLE 5.—*Noguchi system. Syphilis, parasyphilis, hereditary syphilis, and syphilis suspects.*

	Cases examined	+		−	±
		No.	Per cent.		
Primary syphilis	70	65	92.8	4	1
Secondary syphilis	197	190	96	5	2
Tertiary syphilis	177	159	89.9	16	2
Early latent syphilis	115	87	75.6	24	4
Late latent syphilis	150	119	79.3	27	4
Under prolonged treatment	39	4	10.2	32	3
Cerebral syphilis	5	3	60	1	1
Tabes	125	85	68	27	13
General paralysis	15	13	86.6	2	0
Hereditary syphilis	17	17	100	0	0
Syphilis (?)	172	60	34.8	96	16
	1082	802		234	46

TABLE 6A.—*Comparison of the Wassermann and Noguchi systems.*

	Cases examined	Wassermann			Noguchi		
		+		−	+		−
		No.	P. ct.		No.	P. ct.	
Primary syphilis	23	17	73.9	6	20	86.9	3
Secondary syphilis	79	69	87.3	10	76	96.2	3
Tertiary syphilis	65	52	80	13	57	87.6	8
Early latent syphilis	27	13	48	14	18	66.6	9
Late latent syphilis	32	24	75	8	27	84.3	5
Tabes	18	8	44	10	13	72.2	5
	244	183		61	211		33

100 per cent. (Morgenroth and Stertz). The results are uniformly high, especially when contrasted with tabes and cerebrospinal lues (Table 7). In tabes the

TABLE 6B.—*Comparison of the Wassermonn ond Noguchi systems (results obtoined by Howord Fox).*

	Cases examined	Wassermann			Noguchi		
		+		−	+		−
		No.	P. ct.		No.	P. ct.	
Primary syphilis	7	7	100	0	7	100	0
Secondary syphilis	37	36	97	1	37	100	0
Tertiary syphilis	32	23	71	9	27	84	5
Latent syphilis	54	25	46	29	34	62	20
Hereditary syphilis	1	1	..	0	1		0
Tabes	3	3	100	0	3	100	0
Cases for diagnosis	53	21	39	32	26	49	27
Nonsyphilitic cases	23	2	..	21	4*		19
	210						

*One of these four cases has been examined by the writer and reported in his tables, and it was a case of eczema. Subsequent examination of 30 more cases of eczema by the writer came out uniformly negative.

TABLE 6C.—*Comporison of the Wossermonn ond Noguchi systems (results obtoined by D. M. Koplon).*

	Cases examined	Wassermann			Noguchi		
		+		−	+		−
		No.	P. ct.		No.	P. ct.	
Primary syphilis	138	122	90	16	134	97	4
Secondary syphilis	281	242	86	39	270	98	11
Tertiary syphilis	191	140	73	51	155	81	36
Latent syphilis	79	41	51	38	60	75	19
Hereditary syphilis	20	18	90	2	18	90	2
Tabes	205	125	60	80	134	65	71
General paresis	61	40	65	21	44	72	17
Cases for diagnosis	311	98	31	213	180	57	131
	1286	826		460	995		291

figures vary from 54.5 per cent. to 66.6 per cent. In cerebral syphilis the presence of the binding substance is very uncertain; Plaut examined four cases with uniformly negative results.

TABLE 7.—*Cerebrospinal fluids.*

	General paralysis		Tabes		Cerebrospinal syphilis	
	No. of cases	P. ct. +	No. of cases	P. ct. +	No. of cases	P. ct. +
Marie and Levaditi	39	73	9	66.6		
Marie, Levaditi, and Yamanouchi	30	93				
Stertz	45	88.8	5	60	8	0
Noguchi and Moore	60	73	11	54.5	6	50
Wassermann and Plaut	41	88				
Morgenroth and Stertz	8	100				
Plaut	54	90		....	4	0
Nonne	?	90	?	50	16	25
Schütze	..	....	12	66.6		
Marinesco	35	94	15	53		
Smith and Candler	64	92.1				
Noguchi, Rosanoff, and Wiseman	56	87.5				
	432	90	52	56.2	34	19

TABLE 8.—*Examinations of blood-serum and cerebrospinal fluid in cases of leprosy.*

	Serum		Spinal fluid	
	No. of cases	Per cent. +	No. of cases	Per cent. +
Eitner	2	100		
Wechselmann and Meier	1	100		
Slatineanu and Danielopolu	26	100*	19	72
Do	21	57*	20	0
Jundell, Almqvist, and Sandman	26	30*		
Bruck and Gessner	10	50†		
Noguchi	10	70		
	86	72.4	39	36

* Including weak reactions. † Five out of seven cases of tubercular form.

Certain investigators have reported a high percentage of positive reactions in leprosy (as will be seen by perusing Table 8) and in other nonspecific

TABLE 9.—*Cases of scarlatina.*

	No. of cases	P. ct. +		No. of cases	P. ct. +
Much and Eicheberg..	130	46	Seligmann and Klopstok	30**	
Jochmann and Töpfer	33	0	Boas and Hauge.....	61	1.5†
Halberstädter, Müller, and Reiche.....	10	50*	Bruck and Cohn.....	28	0
Meier	52	1.8†	Noguchi.....	63	1.5†
Hoehne.....	37	2.5†	Fùa and Koch.....	57	25§
			Hecht.....	106	1

* Weak reactions only, which gave negative results when tested with several other extracts.

** All negative in 13 cases examined on July 1–3, but 16 additional cases examined one month later with the same antigen gave 3 weak and 13 strong positive reactions. These investigators are inclined to think that their antigen altered on standing, hence the positive results.

† One case showed some inhibition. The case of Noguchi was subsequently proven to be a child with congenital lues.

§ Weak reactions only, which finally disappeared on standing.

diseases, notably scarlet fever (Table 9), carcinoma, and diabetes mellitus.[1] In Table 10 the writer presents a study of the results of 333 cases in which syphilis

[1] A few investigators obtained positive reactions in an astonishingly large proportion of nonsyphilitic cases, while the majority of the workers do not get such results. Among those who reported a large number of positive reactions in nonsyphilitic cases may be mentioned Weil and Braun and Elias, Neubauer, Porges, and Salomon. Weil and Braun encountered 4 positive in 12 cases of pneumonia, 3 positive out of 20 cases of typhoid fever, 2 positive out of 21 cases of tuberculosis, 1 positive out of 4 cases of diabetes mellitus, and 2 positive in 11 cases of tumors. Elias and others found 5 positive in 33 cases of tuberculosis and 4 positive in 14 cases of tumors. Hancken met with 2 positive reactions in 28 control cases, one being a subject with scarlatina and one other with diphtheria. Löhlein examined 250 cases and obtained positive results in 4 cases of tuberculosis and carcinoma. Later investigators, especially those who had been working with the reaction constantly, all failed to get such results as are presented above, if not absolutely free from getting an occasional weak positive reaction in cases of carcinoma, scarlet fever, or diabetes. It should be suspected that when one obtains a high percentage of positive reactions in nonsyphilitic cases he is not doing the test properly.

does not play an etiological part. In Tables 11 and 12 the writer gives the results of examination of 132 cases of diseases in which syphilis may be an etiological factor and of 29 cases of eye diseases of all sorts.

TABLE 10.—*Noguchi system. Cases in which syphilis can be excluded with a fair degree of certainty.*

	Cases examined	+	−	±
Leprosy	10	7	2	1
Carcinoma	51	1	48	2
Sarcoma	3	0	3	0
Adenosarcoma	1	0	1	0
Endothelioma	1	1	0	0
Scarlatina	63	1	60	2
Varicella	1	0	1	0
Measles	2	0	2	0
Tuberculosis	52	0	52	0
Lupus	2	0	2	0
Banti's disease	1	1	0	0
Hodgkin's disease	2	0	2	0
Muscular distrophy	5	1	3	1
Neurasthenia	2	0	1	1
Dementia præcox	5	0	5	0
Various skin diseases	58	0	57	1
Miscellaneous	74	0	74	0
	333	12	313	8

In certain nervous diseases of unknown origin the Wassermann reaction has been resorted to as a means of determining, if possible, the nature of the causative factor. Thus, Raviart, Breton and Petit examined various forms of insanity, aside from parasyphilitic patients, in regard to the presence of this

TABLE 11.—*Noguchi system. Cases in which syphilis is an etiological factor or cannot be excluded as a possible cause of the condition.*

	Cases examined	+	—	±
Cirrhosis of liver	7	5	1	1
Ascitic fluids	21	11	9	0
Aortic insufficiency	1	1	0	0
Chronic arthritis	10	2	6	2
Eye cases	29	14	15	0
Diabetes	5	1	4	0
Eczema	32	1*	31	0
Scleroderma	4	1	3	0
Brain tumor (?)	8	4	4	0
Central gliosis (?)	2	1	1	0
Hemiplegia	8	3	5	0
Spastic paraplegia	3	2	0	1
Raynaud's disease †	2	0	2	0
	132	46	81	4

*This case has been reported also by Fox, in Table 6B. For the other 31 cases I am indebted to Dr. Daisy Orleman Robinson.

†Kaliski and Buerger, using Wassermann's and Noguchi's systems, got negative results in 16 cases of thrombo-angeitis obliterans.

TABLE 12.—*Noguchi system. Cases of eye diseases.**

	Cases examined	+	—	±
Keratis interstialis	12	8	4	0
Iritis	6	4	2	0
Scleritis	1	0	1	0
Paralysis external rectus	1	0	1	0
Optic neuritis	1	1	0	0
Choroiditis exudat	1	0	1	0
Optic atrophy	5	0	5	0
Acromegaly with ocular symptoms	2	1	1	0
	29	14	15	0

*For the clinical material I am indebted to Dr. Martin Cohen.

reaction in the blood. Their results are somewhat striking, as they got positive reactions in about 30 to 40 per cent. of cases of epilepsy, idiocy, and im-

becility. In three of five cases of dementia senilis and in five out of 19 cases of dementia præcox they got positive results. Raubinovitch and Levaditi examined 15 cases of dementia præcox and got positive

TABLE 13.—*Psychiatric cases.*

Clinical diagnosis	Blood serum				Cerebrospinal fluid				Syphilis ascertained
	No. of cases.	Reactions			No. of cases.	Reactions			
		—	+	±		—	+	±	
Arteriosclerotic dementia	10	10	0	0	9	9	0	0	
Brain tumor	1	1	0	0	1	1	0	0	
Traumatic psychosis	1	1	0	0	1	1	0	0	
Senile dementia	16	13	1	2	10	8	1	1	
Infant. cerebr. palsy	6	6	0	0	5	5	0	0	
Epilepsy	69	48	12	9	55	50	3	2	
Huntington's chorea	2	1	1	0	1	1	0	0	
Uræmic psychosis	1	1	0	0	1	1	0	0	
Alcoholic psychosis	9	4	2	3	6	4	1	1	4
Polyneuritic psychosis	8	7	1	0	8	8	0	0	
Involution melancholia	10	8	2	0	7	7	0	0	
Dementia præcox	131	99	15	17	83	76	3	4	5
Manic depressive insanity	14	9	2	3	7	5	2	0	1
Paranoic condition	9	7	1	1	4	4	0	0	
Imbecility	6	4	2	0	6	6	0	0	
Constitutional inferiority	1	1	0	0	1	1	0	0	
Unclassified	40	28	6	6	38	35	2	1	5
	334	248	45*	41*	243	222	12*	9*	15

*These cases showing positive and doubtful reactions may have had syphilis, but it was difficult to ascertain the disease in all the cases. In 15 cases at least, syphilis was proven to be present.

results in 20 per cent. with the blood, but all the spinal fluids examined gave negative results. This last fact agrees with the observation of the writer and Moore, Rosanoff, Wiseman, and the writer examined 413 cases of various forms of insanity for the reaction

in serum and cerebrospinal fluid and obtained the results similar to those of previous investigators.

In gynæcological conditions the reaction has also been called upon to test the validity of the laws of Colles and Profeta. Müller found that with the blood of wives of syphilitic husbands, where the former had repeated abortions and premature births, the results were usually negative and that their offspring also gave negative reaction. Knöpfelmacher and Lehndorffer examined 32 apparently healthy mothers of syphilitic children and obtained positive reactions in 18. Halberstädter, Müller, and Reiche found that the reaction may be negative with children of syphilitic mothers, and *vice versa,* while Boas and Thomsen assert that the reaction can develop later in children whose blood gives a negative result at the time of birth. They all agree that the negative reaction in these children or mothers is largely due to the latency of the disease, but is not a sign of immunity against the disease. Thus, while the mother of a syphilitic infant may present no sign of syphilis, yet examination of the blood of the mother gives positive reaction in half the number of cases examined. However, much more has to be done before the dictum of Colles can be overthrown.

EFFECT OF TREATMENT UPON THE REACTION.

Much work has been done by numerous investigators to determine the result of various forms of treatment upon the syphilitic antibodies in the blood, and it would seem that the time has not yet come to make a dogmatic statement upon this subject. It is known, however, that the reaction frequently disappears after a short course of treatment, as will be pointed out below, often to return again within a greater or lesser period of time.

Citron, who was among the first to investigate the effect of treatment upon the reaction, found that whereas before treatment the percentage of positive results obtained by him was 81, treatment had the effect of reducing the figures to 65 per cent. In about half of these cases, numbering 57 in all, but one course of treatment was given. Bruck and Stern obtained positive reactions in 81.5 per cent. of 173 untreated cases, and in another group of treated cases got positive reactions in only 28 per cent. Blaschko studied 52 positive cases of manifest syphilis and after treatment 45 of these gave negative results; of 38 cases of latent syphilis 31 gave negative results after treatment. Hoehne studied 211 cases which before treatment gave positive reactions, and found that in 56 per cent. the reaction disappeared after therapeutic interference, but in spite of some treatment 33.9 per cent. gave a positive reaction. In five cases,

after eleven to twelve injections of mercuric salicylate over a period of two months, the reaction was positive. Lesser states that a positive reaction can be made negative in about 35 per cent. of cases by giving 30 inunctions of mercury, 12 injections of an insoluble mercuric preparation, or 25 injections of a soluble mercuric preparation. The rapidity with which the reaction disappears is very variable in different individuals. Boas found that after a course of injections over two or three months the reaction became negative in 76 out of 82 cases, and states that the reaction may return within a month after cessation of treatment, indicating a recurrence.

From the above it will be seen that the reaction is affected greatly by the treatment of the disease, but that some cases frequently persist in giving a positive reaction in spite of what is done for them. In hereditary lues the reaction is difficult to get rid of, often persisting in spite of most rigorous interference. The reaction may return shortly after cessation of treatment, so that it may be necessary to make frequent tests to determine whether further therapeusis is indicated. While it seems settled among the profession that a positive reaction in a syphilitic case is an indication for additional treatment, it is not definitely established that the disappearance of the reaction is justification for the cessation of treatment, especially as the reaction may be quickly affected by treatment.

XII.

THE BUTYRIC ACID TEST.

While studying the relation of protein lipoids and salts to the Wassermann reaction, I observed that the syphilitic antibody is contained in or precipitated with globulin, and particularly the euglobulin fraction of the blood-serum or cerebrospinal fluid. I incidentally ascertained that the globulin fraction of these fluids is increased in syphilis, and that there exists a parallel between the titre of the syphilitic antibody and the amount of the globulin fractions, to which rule I observed certain exceptions, in which this parallelism was absent. There would therefore appear to be no necessary connection between the syphilitic antibody and the increase of globulin, although the two conditions are likely to be associated. While the appearance of the antibody and the increase of the globulin are often found associated, I have observed that the increase in the globulin is recognizable earlier than the presence of the antibody; and in the early stages of primary syphilis, when the presence of the antibody may not be detectable, the globulin content is seen already to be increased. Again, in cases of latent syphilis the anti-

body so far as it is demonstrable is more or less inconstant and may thus escape detection altogether, whereas it is exceptional not to find the globulin increased. These facts apply also to the cerebrospinal fluid. In specimens of cerebrospinal fluid coming from cases of secondary or tertiary syphilis in which there is no special involvement of the central nervous system, the syphilitic antibody is extremely difficult to detect, and this is true even though this fluid contains an increase in its protein fraction. In cases of parasyphilitic affections, where the central nervous system is primarily involved, the detection of the antibody is often readily accomplished. In general paralysis, the antibody is detectable in the cerebrospinal fluid in about 90 per cent. of the cases. In locomotor ataxia or cerebral or spinal syphilis, the detection of the antibody in the cerebrospinal fluid is successful in about 60 per cent. of the cases. The increase of protein in the cerebrospinal fluid of these cases is greater than the appearance of the antibody, and my experience leads me to conclude that the abnormally high protein content is a more constant occurrence in the cerebrospinal fluid in syphilitic and parasyphilitic cases than is the presence of a detectable antibody.

For the detection of the increase of globulin in the blood or cerebrospinal fluid numerous methods have been devised by different investigators. For

the estimation of the globulin of the blood, the methods proposed are the usual chemical procedures, the application of which to clinical laboratories has not been successfully accomplished. The detection of the increased globulin in the cerebrospinal fluid has been accomplished by simpler quantitative methods, such as the half saturation with ammonium sulphate, as devised by Nonne and Apelt, which is applicable to clinical laboratories. On the whole, this method, while useful, is somewhat difficult of application and fails to give a proper differentiation when the increase in the globulin is only slight. My own method consists in the employment of butyric acid as precipitant for the globulin, but the manner of application differs somewhat according as it is applied to the blood-serum or the cerebrospinal fluid. This method of detecting an increase in the globulin content is applicable especially to the cerebrospinal fluid, and is so simple as to be within the reach of the simplest laboratory.

Method for Cerebrospinal Fluid.—One or two parts of the cerebrospinal fluid to be examined are mixed with 5 parts of a 10 per cent. butyric acid solution in physiological salt solution, and are heated over a flame and boiled for a brief period. One part of a normal solution of NaOH is then added quickly to the heated mixture, and the whole boiled once more for a few seconds. The actual quantities of

these three agents that I prefer are 0.1 or 0.2 c.c. of the spinal fluid, 0.5 c.c. of the butyric acid solution, and 0.1 c.c. of the normal sodium hydroxide. It is necessary to take the precaution to employ for this test only cerebrospinal fluid entirely free from blood.

The presence of an increased content of protein in the cerebrospinal fluid is indicated by the appearance of a granular or floccular precipitate, which gradually settles to the bottom of the tube, beneath a clear, supernatant fluid. The velocity and intensity of the reaction vary according to the quantity of the protein contained in a given specimen. The greater the amount of the protein, the more quickly and distinctly the reaction appears. The granular precipitate appears within a few minutes in a specimen containing a considerable increase in protein, while two hours may be required to obtain a distinct reaction in specimens weaker in protein. In obtaining the reaction, the time period should not be greater than two hours.

This reaction I have found to appear regularly in the cerebrospinal fluid of the patients with syphilitic and parasyphilitic affections, and also in all cases of inflammation of the meninges caused by such micro-organisms as *Diplococcus intracellularis,* pneumococcus, influenza bacillus, tubercle bacillus, etc.

These acute inflammatory infections are of course readily differentiated from the syphilitic affections. Normal cerebrospinal fluid gives with the butyric acid test a slight opalescence and sometimes a marked turbidity, but the granular precipitate does not occur at all or occurs only after several hours or even after twenty-four hours.

Method for Blood-serum.—One part of clear serum free from hæmoglobin is mixed with 9 parts of a half-saturated solution of ammonium sulphate, the precipitated globulin centrifugalized by a powerful machine, and the compact globulin fraction separated by decantation from the supernatant fluid. The deposit may now be weighed to obtain an idea of its quantity. It is then redissolved in 10 parts of 0.9 per cent. salt solution, and is ready for the test. The test is made by mixing one part of the solution with an equal part of 10 per cent. butyric acid solution, when a prompt, dense, milky turbidity appears in the mixture, if the serum tested was derived from cases of syphilis, while it remains clear or shows only a slight opalescence without precipitation after several hours' standing, if it was derived from persons not suffering with syphilis.

In carrying out this test I have been in the custom of using 0.5 c.c. of serum and 4.5 c.c. of ammonium sulphate solution, and of performing the centrifu-

galization for 30 minutes in a machine which runs at the rate of 5000 revolutions per minute. After decanting the fluid, the deposit is redissolved in 5 c.c. of 0.9 per cent. salt solution. Of this solution, 0.5 c.c. is mixed with an equal quantity of the butyric acid solution. It is advisable to carry out the examination of the several specimens of the fluid at the same time, and especially to include a normal serum, giving a negative reaction, to act as a control for the series.

It should be mentioned that the weights of the globulin deposits are comparable with one another only when they are packed equally by a definite degree of centrifugalization. Under similar conditions the use of weighing to determine the increase in the globulin is an advantage, but where the conditions are not identical, such weighings may give an erroneous indication.

BUTYRIC ACID REACTION OF CEREBROSPINAL FLUID IN SYPHILIS AND PARASYPHILITIC DISEASES.

The results which I have obtained with the butyric acid reaction, along with Dr. Moore of the Manhattan State Hospital on Ward's Island, will serve to illustrate the value of the butyric acid test in psychiatry. For the purpose of the test the cerebrospinal fluid from cases of general

TABLE 14.—*Results in cases in which the diagnosis was reasonably certain.*

Cases	No. of cases	Butyric acid reaction			Wassermann reaction			Cell count		
		+	—	±	+	—	±	+	—	±
Syphilis:										
Secondary stage... (without nervous symptoms)	3	3	0	0	0	3	0	0	3	0
Tertiary stage..... (without nervous symptoms)	1	1	0	0	0	1	0	0	1	0
Cerebral syphilis..	3	3	0	0	1	1	1	3	0	0
Spinal syphilis....	3	3	0	0	2	1	0	3	0	0
Hereditary syphilis	10	9	0	1	8	2	0			
Parasyphilis										
General paralysis:										
Cerebral.....	43	37	4	2	32	6	5	39	2	2
Tabetic.....	17	17	0	0	12	3	2	16	1	0
Tabes.....	11	11	0	0	6	4	1	11	0	0
	91	84	4	3	61	21	9			
Psychoses:										
Arteriosclerotic ...	3	1	2	0	1	2	0	1	2	0
Traumatic.....	2	0	2	0	0	2	0	0	2	0
Senile.....	1	0	1	0	0	1	0	0	1	0
Epileptic.....	6	0	6	0	0	5	1	0	6	0
Alcoholic.....	7	0	6	1	3	3	1	0	6	1
Manic-depressive.	2	0	2	0	1	1	0	0	2	0
Dementia præcox .	11	1	10	0	1	8	2	1	10	0
Imbecility.....	2	0	2	0	0	2	0	0	2	0
	34	2	31	1	6	24	4	2	31	1

paralysis, tabes dorsalis, dementia præcox, epilepsy, alcoholic psychosis, senile dementia, and certain other forms of insanity was employed. In order to arrive at an accurate result, the fluids tested by means of the butyric acid were also subjected to the

Wassermann test and to the usual cytodiagnosis observation. In order to make the series complete, the cerebrospinal fluid from several cases of syphilis free from involvement of the central nervous system was also examined.

TABLE 15.—*Analysis of the reactions with regard to syphilis.*

Cases	No. of cases	Butyric acid reaction			Wassermann reaction			Cell count		
		+	—	±	+	—	±	+	—	±
General paralysis and Tabes:										
Syphilis +	36	34	1	1	26	8	2	36	0	0
Syphilis—	16	13	3	1	10	3	3	11	3	2
Other diseases:										
Syphilis +	1	1	0	0	1	0	0	1	0	0
Syphilis —	12	1	11	0	3	8	1	1	11	0

In the secondary and tertiary stages of syphilis, without direct involvement of the nervous system, the cerebrospinal fluid yielded a reaction of feeble intensity to the butyric acid test. These fluids gave neither a positive cytodiagnosis nor the Wassermann reaction. The cerebrospinal fluid of a group of cases of hereditary syphilis gave a positive butyric acid reaction in about 90 per cent. and a positive Wassermann reaction in about 80 per cent. of those examined. On the other hand the cerebrospinal fluid obtained from cases of cerebral and spinal syphilis yielded the butyric acid reaction in all cases and at the same

time gave a positive cytodiagnosis. As against these results is to be placed the result with the Wassermann reaction, which was positive in 50 per cent. of the cases examined. The cerebrospinal fluid obtained from cases of general paralysis gave positive butyric acid reaction in 90 per cent., positive cytodiagnosis in 91 per cent., and positive Wassermann reaction in 73 per cent. of those examined. The cerebrospinal fluid from cases of tabes dorsalis gave positive butyric acid reaction and cytodiagnosis in all, or 100 per cent., and positive Wassermann reactions in 53 per cent. of those examined. Finally, the cerebrospinal fluid obtained from patients suffering with various forms of psychosis in whom a syphilitic history was excludable, or at least not obtained, gave positive butyric acid reactions and cytodiagnosis in 2.8 per cent. and positive Wassermann reactions in 13 per cent. of those examined.

From the above statement it becomes at once evident that the butyric acid reaction runs parallel with the cytodiagnosis in cases of parasyphilitic disease, and is especially reliable as an indicator of that condition, with the cytodiagnosis. Moreover, the results of the examination of the cerebrospinal fluid of cases of secondary and tertiary syphilis, in which there are no special lesions of the central nervous system, indicates through the feeble reaction obtained that a

protein increase in the fluid is not necessarily associated with an increase and change in the number and quality of the cells contained in the fluid. In other words, the butyric acid reaction not only detects the changes in the fluid associated with parasyphilitic disease and with direct syphilitic lesions of the central nervous system, but it also indicates the existence of a general syphilitic state of infection, which cannot be detected by the means of cytodiagnosis.

The butyric acid reaction, as stated, is about parallel in results with cytodiagnosis, but is not parallel with the results of the Wassermann reaction. In cases with established syphilitic history, the butyric acid reaction gives a higher percentage of positive result than the Wassermann reaction. It is therefore somewhat confusing to find that in nonsyphilitic forms of psychosis, the percentage of positive result of the Wassermann reaction exceeds that of the butyric acid test or cytodiagnosis. This discrepancy has not been cleared up, and calls for further study. Whether or not it has to do with the existence of an independent constituent similar to syphilitic "antibody" in the cerebrospinal fluid in these cases needs determination.

It is obvious that the butyric acid test is a useful addition to our diagnostic methods in the detection of parasyphilitic diseases of the central nervous system, and of cerebrospinal syphilis. It is, of

course, desirable to confirm as often as possible the indications of the reactions by post-mortem examinations. Of the series which we examined, 17 cases have come to autopsy. Of these, 15 had given positive butyric acid tests, 14 having been diagnosed as cases of general paralysis, and one as a case of cerebral syphilis. Two had given negative tests. The autopsy findings were in complete agreement with the indications of the test.

In a series of investigations recently conducted at the Kings Park State Hospital, New York, Rosanoff, Wiseman, and the writer confirmed and extended the observations of Moore and the writer referred to above. In all 413 cases have been examined from the standpoints of the Wassermann reaction, butyric acid test, and cytodiagnosis. Of this total number 252 cases were available for the four tests simultaneously (the Wassermann reaction in serum and in cerebrospinal fluid, butyric acid test, and cytodiagnosis), while in the remaining 161 cases the examination of the spinal fluid was either incomplete or not undertaken. The Wassermann reaction was done by the writer's system [1] and is designated in the tables as W-N. In Table 16 the results obtained with general paralysis are presented. It is interesting to notice that the increase of protein and of lymphocytes in cerebrospinal fluid of these cases is distinctly more

[1] See Chapter VII, page 47.

TABLE 16.—*General paralysis.*

Variety of test		Serum	Cerebrospinal fluid			No. of cases for each group	Frequency of association of different reactions analysed		
		W.-N.	W.-N.	Butyric acid	Cells				
Grouping of cases according to the frequency and mode of combination of the four different reactions for which they were examined.		+	+	+	+	24	Four reactions all positive:— 31 = 70%	Cases with more than three reactions positive:— 38 = 86.4%	Cases with more than two reactions positive:— 44 = 100%
		±	+	+	+	1			
		+	±	+	+	2			
		±	±	+	+	4			
		—	+	+	+	4	Only three reactions positive:— 7 = 16.4%		
		+	+	+	±	1			
		±	—	+	+	1			
		±	+	+	—	1			
		—	+	+	—?	1	Only two reactions positive:— 6 = 13.6%		
		—	+	—?	+	1			
		—	—	+	+	4			
Number and percentage of positive and negative reactions obtained in corresponding tests.	+	27 = 62%	32 = 72.8%	43 = 97.7%	41 = 93.2%	44			
	±	7 = 16.1%	6 = 13.6%	0	1 = 2.3%				
	—	10 = 22%	6 = 13.6%	1 = 2.3%	2 = 4.5%				
	+ and ±	78%	86.4%	97.7%	95.5%				

Syphilis ascertained in..... 16 cases
Syphilis negative in ..... 9 cases
Syphilis unascertainable in. 19 cases
44 cases

TABLE 17A.

		Serum	Cerebrospinal fluid			No. of cases in each group	Remarks
		W.-N.	W.-N.	B.	Cells		
Dementia præcox 71 cases		—	—	—	—	45	Syphilis ascertained in 5 cases all reacting positively to the fixation and butyric acid tests. The fixation reaction was present either singly in serum or cerebrospinal fluid or in both. In remaining positive cases syphilis was unascertainable and cannot be excluded. None showed pleocytosis.
		±	—	—	—	4	
		+	—	—	—	9	
		—	+	—	—	2	
		—	±	—	—	3	
		±	—	±	—	2	
		±	±	—	—	1	
		+	—	±	—	4	
		+	+	—	—	1	
	—	50 = 70%	64 = 88.8%	65 = 91.5%	71 = 100%	71	
	±	7 = 10%	4 = 6.6%	6 = 8.5%	0		
	+	14 = 20%	3 = 4.6%	0	0		
Epilepsy 51 cases		—	—	—	—	36	Syphilis unascertainable in this group of cases, but cannot be excluded from the cases giving positive reactions for the fixation and butyric acid tests. None showed pleocytosis.
		±	—	—	—	2	
		—	±	—	—	1	
		+	—	—	—	8	
		—	—	+	—	1	
		±	—	+	—	1	
		+	+	—	—	2	
	—	38 = 74%	48 = 94%	49 = 96%	51 = 100%	51	
	±	3 = 6%	1 = 2%	0	0		
	+	10 = 20%	2 = 4%	2 = 4%	0		

constant than the presence of the Wassermann reaction. This last reaction was more frequently met with in the spinal fluid than in the serum. Note-

TABLE 17B.

		Serum	Cerebrospinal fluid			No. of cases in each group	Remarks
		W.-N.	W.-N.	B.	Cells		
Senile dementia . 9 cases		—	—	—	—	7	Syphilis probable in cases giving positive reactions for the fixation and butyric acid tests, but difficult to establish with certainty. None showed pleocytosis.
		±	—	—	—	1	
		—	+	+	—	1	
	—	8 = 88.9%	8 = 88.9%	8 = 88.9%	9 = 100%	9	
	±	1 = 11.1%	0	0	0		
	+	0	1 = 11.1%	1 = 11.1%	0		
Manic depressive insanity, 6 cases		—	—	—	—	3	Syphilis unascertainable, but possible in positive cases. None showed pleocytosis.
		±	—	—	—	2	
		+	+	—	—	1	
	—	3	5	6	6	6	
	±	2	0	0	0		
	+	1	1	0	0		
Alcoholic psychosis, 4 cases		±	—	—	—	1	Syphilis ascertained in all cases giving positive reactions for the fixation or butyric acid tests. None showed pleocytosis.
		±	—	±	—	1	
		+	—	+	—	1	
		—	+	±	—	1	
	—	1	3	1	4	4	
	±	2	0	2	0		
	+	1	1	1	0		

worthy is it also that the four reactions are present in 70 per cent. of the cases examined and that all gave positive reactions to at least two different tests. This simultaneous presence of positive reactions to more than two different tests is quite characteristic

of parasyphilitic conditions, for it is very seldom that other forms of psychoses give plural positive reactions without a definite syphilitic infection. In Tables 17 A, B, C, D, I present the results obtained with

TABLE 17C.

		Serum	Cerebrospinal fluid			No. of cases in each group	Remarks
		W.-N.	W.-N.	B.	Cells		
Polyneuritic psychosis, 7 cases	—	—	—	—	—	6	Syphilis unascertainable in the case reacting positively to the fixation test in serum. The fixation test in spinal fluid, butyric acid test and pleocytosis negative in all.
	+	+	—	—	—	1	
	—	6	7	7	7	7	
	+	1	0	0	0		
Involution melancholia, 7 cases	—	—	—	—	—	6	Syphilis unascertained in the positive case. All reacted negatively to the fixation test in spinal fluid, butyric acid test and cytodiagnosis.
	+	+	—	—	—	1	
	—	6	7	7	7	7	
	+	1	0	0	0		
Paranoic condition, 4 cases	—	—	—	—	—	3	Syphilis unascertainable.
	+	+	—	—	—	1	
	—	3	4	4	4	4	
	+	1	0	0	0		
Imbecility ..... 4 cases	—	—	—	—	—	2	Syphilis unascertainable.
	+	+	—	—	—	2	
	—	2	4	4	4	4	
	+	2	0	0	0		

other forms of insanity. There are a number of cases giving solitary positive Wassermann reaction, but scarcely any that gave positive cytodiagnosis. Again, we find that the butyric acid test was positive

in some cases with syphilitic histories and the Wassermann reaction in serum was also positive in these cases.

TABLE 17D.

	Serum	Cerebrospinal fluid			No. of cases in each group	Remarks
	W.-N.	W.-N.	B.	Cells		
Infantile cerebral paralysis, 4 cases	—	—	—	—	4	Syphilis unascertained. All reacted negatively.
Arteriosclerotic dementia, 7 cases	—	—	—	—	7	Syphilis unascertained. All reacted negatively.
Brain tumor ..... 1 case	—	—	—	—	1	Syphilis unascertained. All reacted negatively.
Traumatic psychosis, 1 case	—	—	—	—	1	Syphilis unascertained. All reacted negatively.
Unclassified .... 32 cases	—	—	—	—	18	Syphilis ascertained in 5 cases reacting positively either to the fixation or to the butyric acid test or to both. In other positive cases it was unascertainable and unexcludable. None showed pleocytosis.
	±	—	—	—	4	
	+	—	—	—	3	
	—	—	±	—	2	
	—	—	+	—	2	
	+	—	+	—	1	
	±	+	—	—	1	
	±	—	±	—	1	

BUTYRIC ACID REACTION IN GENERAL DISEASES.

That the butyric acid reaction will serve a useful purpose in the diagnosis of nervous and mental diseases has not only been indicated by my own studies in conjunction with Dr. Moore, but by several reports from other sources, in which the reaction has been applied. It is, however, necessary that the limit of

the test should be precisely defined, which can be done by subjecting the cerebrospinal fluid from a large number of general diseases to the action of the test. It will require such a wide study to determine whether or not the reaction is either specific or special for syphilitic disease in a manner to render it useful for diagnosis. It can, of course, be predicted that in all cases in which there is increase in protein, and particularly in the globulin fraction of the cerebrospinal fluid, the reaction will be given. Now there are other diseases which produce this increase of protein in the cerebrospinal fluid, and they would naturally yield the reaction. The chief if not the sole diseases other than syphilis in some of its stages, associated with this exudative inflammatory condition, are the acute infections of the cerebrospinal meninges. Thus in all acute and subacute inflammations of the meninges a positive reaction is obtained. Luckily, there is no difficulty whatever likely to be experienced in the separation of this class of cases, as has already been mentioned (see page 119). The only example of inflammation which might be confused with a syphilitic affection is tubercular meningitis, because, in this affection, the fluid is clear and contains an excessive number of mononuclear cells. But this affection is distinguishable readily not only by the clinical history, but also by the presence of the tubercle bacilli.

Should, however, cases arise in which there remains some doubt, this can be eliminated readily by invoking the aid of the Wassermann reaction, in one of its forms. The cerebrospinal fluid in typhoid fever and pneumonia, independent of the acute inflammations of the meninges which sometimes attend these diseases, do not yield the butyric acid reaction.

The butyric acid reaction will, I believe, suffice to distinguish normal from pathological cerebrospinal fluid, and especially that form of pathological fluid which is altered through an increase in its protein constituent. It may therefore prove applicable to some of the ill-defined inflammatory conditions of the meninges—such, for example, as the so-called serous meningitis, in which condition the micro-organisms and inflammatory cells are not, as a rule, demonstrable. If the excessive serous exudation differs from the normal cerebrospinal fluid in the manner which is characteristic of inflammatory exudates, the butyric acid reaction would be obtainable. It also suggests itself that the reaction would be with profit invoked in certain cases of tubercular meningitis in which tubercle bacilli are not readily demonstrable. A positive reaction with butyric acid will, of course, be given by the cerebrospinal fluid provided there is tubercular inflammation, and the same fluid will be negative to the Wassermann test. Hence the two tests, together with the clinical history, may lead to

a provisional diagnosis at a time when the tubercle bacilli have not yet been discovered and animal inoculations have not yet had time to declare the nature of the disease.

TABLE 18.—*The butyric acid reaction in general diseases.*

Cases	No. of cases	Butyric acid reaction			Wassermann reaction		
		+	—	±	+	—	±
Diseases of the meninges:							
Epidemic cerebrospinal meningitis	14	14	0	0	0	14	0
Pneumococcal meningitis	6	6	0	0	0	6	0
Influenzal meningitis	1	1	0	0	0	1	0
Tubercular meningitis	30	30	0	0	0	30	0
Hydrocephalus externus	2	2	0	0	1	1	0
	53	53	0	0	1	52	0
Diseases without meningeal involvement:							
Typhoid fever	1	0	1	0	0	1	0
Pneumonia	4	0	4	0	0	4	0
Pulmonary tuberculosis	1	0	1	0	0	1	0
Enterocolitis	2	0	2	0	0	2	0
Rhachitis	1	0	1	0	0	1	0
Uræmiæ	2	0	2	0	0	2	0
Septicæmia	1	0	1	0	0	1	0
Miscellaneous without nervous involvement	12	0	11	1	0	10	2
	24	0	23	1	0	22	2

BLOOD-SERUM.

I have subjected about 300 specimens of blood-serum, taken from cases of syphilis, from normal persons, and from persons suffering with

other diseases than syphilis, to the globulin estimation by means of the butyric acid precipitation and by direct weighing. According to my observations, the globulin content of normal blood-serum varies from 0.120 to 0.150 gram per 0.5 c.c. of the serum, weighed in the moist condition. The weight of the dry globulin is about one-eighth to one-ninth of that of the moist specimens. The blood-serum which contains normal globulin content, prepared in the manner described (see page 120), does not give a positive butyric acid reaction.

In cases of untreated and manifest secondary and tertiary syphilis, the globulin content of the serum, weighed in the moist condition, varies from 0.200 to 0.350 gram per 0.5 c.c. In the primary stages of syphilis the increase in globulin is less great, but is still sufficiently pronounced to give the butyric acid reaction. In cases of latent syphilis, the globulin content rarely exceeds 0.200 gram and may be somewhat less, but is also sufficiently increased to give the reactions. On the other hand, cases of syphilis which have been well treated and have not exhibited symptoms for many years, showed no increase in the globulin content over that of normal individuals.

I have therefore studied cases of syphilis in progress of treatment, and have found that, as the treat-

ment progresses and the symptoms disappear, the reaction becomes less and less pronounced, and that the globulin tends correspondingly to approach closer and closer the normal quantity. However, the butyric acid reaction does not entirely disappear from the serum for many months after treatment has been carried on and the obvious symptoms have entirely disappeared. As a rule, the butyric acid reaction disappears later than the Wassermann reaction, and the intensity of the butyric acid reaction does not always run parallel with that of the Wassermann reaction.

I have, of course, studied the blood-serum derived from persons suffering from diseases of a general nature, in whom syphilis could be excluded. It is important to record that, in a small number of cases of carcinoma and tuberculosis, the butyric acid reaction was obtained, while the Wassermann reactions were negative. Similarly, two cases of Hodgkin's disease which I examined gave strong butyric acid reactions, but no Wassermann reaction. It has been pointed out by Gay and Fitzgerald that in some acute infectious diseases, such as pneumonia and scarlet fever, there may occur an increase in the globulin content leading to a positive butyric acid reaction. With these facts in mind, it is not difficult to define the limit of application of the butyric acid reaction to blood-serum.

It may therefore be stated that the reaction is not specific, and when it is present it does not necessarily indicate a syphilitic infection. But, on the other hand, it can be employed to establish or confirm a deduction based upon the clinical history and the results of the Wassermann reaction and thus become indirectly of diagnostic value. On the other hand, a negative result is valuable as excluding a syphilitic infection. In this respect the reaction has advantages over the Wassermann reaction, in which a negative result is not always reliable as indicating absence of syphilitic infection.

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GLOSSARY.

Agglutination.

Clumping of bacteria or blood corpuscles by specific agglutinins. For corpuscles the term hæmagglutination is often used.

Agglutinins.

A single or repeated injection of bacteria or foreign blood corpuscles into an animal is followed by the development of a new property in the serum of that animal. This serum, when deprived of its own complement either by inactivation or by dilution, is capable of clumping in test tube the bacteria or blood corpuscles employed for immunization. This phenomenon is called agglutination and is ascribed to the reaction product designated agglutinin. Its nature is not known except that it is found in the protein fraction of serum and resists the temperature of 56°C. Agglutinins may be found in some normal sera in varying quantity. Agglutinins being antibodies are specific, and can be absorbed by bacteria or blood-corpuscles.

Alexin.

First introduced by Hans Buchner, adopted by Bordet; is now synonymous with complement of Ehrlich and cytase of Metchnikoff. See complement. Buchner's idea of alexin is not identical with that of Bordet, and the term was used to designate bacteriolysins and hæmolysins, but not complement of Ehrlich or alexin of Bordet. The term alexin to-day is used in Bordet's sense but not in Buchner's.

Amboceptor.

Introduced by Ehrlich; is synonymous with Fixateur of Metchnikoff Substance sensibilisatrice of Bordet, Preparator of Max Gruber, and Copula of P. Th. Müller. Amboceptor is one of the two active principles necessary to cause Hæmolysis, Bacteriolysis, or any other cytolysis caused by serum, the other active principle being complement. Amboceptor retains its activity after the serum is heated to from 55° to 56° C. for 30 minutes, while complement is destroyed at that temperature. Amboceptor, as well as complement, is present in the coagulable protein fraction of serum. Amboceptor may be present in any normal serum, and can be produced in the serum of an animal by injecting repeatedly the cells for which it has no amboceptor. The amboceptor normally present is called natural amboceptor and that which is produced by means of repeated injections of foreign cells is called immune amboceptor. The amboceptor capable of causing hæmolysis (in presence of complement, of course) is called hæmolytic amboceptor, while that which is capable of dissolving bacteria is called bacteriolytic amboceptor. A few writers use the simple terms of hæmolysin or bacteriolysin instead of hæmolytic or bacteriolytic amboceptor. Amboceptors are capable of producing anti-amboceptors when injected into a susceptible animal.

Antibodies.

A general term applied to a group of reaction products arising from single or repeated administrations of antigens to a suitable animal. Immune body is a synonym of antibody. Among antibodies we may enumerate hæmolytic amboceptors, bacteriolytic amboceptors, other cytolytic amboceptors, precipitins, agglutinins, antitoxins, antivenins, antiricin, antiabrin, etc. Antibodies possess specific affinity for the antigens which are used for their production. Certain antibodies such as agglutinins, amboceptors, antitoxins, or antihæmolysins may be normally present in certain sera in small amount. A group of antibodies is capable of producing antibodies when injected into another animal, thus forming anti-antibodies.

Anticomplementary action.

Substances possessing the power of reducing or removing totally the action of complements are said to be anticomplementary. Most acids, alkalies, and certain salts have anticomplementary action. In certain sera there are often certain principles possessing anticomplementary properties. Human serum gradually acquires this action on standing. Repeated injections of fresh serum into an animal of another species is followed by the appearance of anticomplements (Ehrlich and Morgenroth), while Gay considers it as an example of complement-fixation by specific precipitate.

Antigens.

A general term applied to a group of substances capable of producing specific antibodies administered once or repeatedly, usually by injection, to a suitable animal. For example, bacteria, blood corpuscles, and certain somatic cells are antigens because they produce specific antibodies called amboceptors and agglutinins. Blood serum, milk or bacterial extracts are also antigens, because they produce antibodies called precipitins, each being specific for the substance employed for its production. On the other hand, most inorganic or organic substances with definite chemical structure are not antigens, because their introduction is not followed by the formation of antagonistic substances (antibodies) in the body. Repeated administrations of various alkaloids render the organism gradually more resistant to their effect, but do not produce antibodies, hence these alkaloids are not antigens. Diphtheria toxin, tetanus toxin, ricin, abrin, snake venoms, are antigens and their injections are followed by specific antitoxins, as is well known.

Bacteriolysins.

Active principles in blood serum capable of dissolving bacteria, consist of specific bacteriolytic amboceptors and complement. Analogous to hæmolysins and cytolysins in general.

Bacteriolysis.

Dissolution of bacteria by immune or normal sera. It is caused by specific bacteriolytic amboceptors and complement. Analogous to hæmolysis.

Bacteriotropins.

Introduced by Neufeld; are active principles of certain immune sera inducing phagocytosis. Their action is on the bacteria but not on the phagoctyes. They are thermostable.

Complement.

Introduced by Ehrlich; is synonymous with Metchnikoff's cytase and Bordet's alexin. By the term complement one understands one of the two active principles concerned in Hæmolysis, Bacteriolysis, and other instances of serum cytolysis. The other principle is called amboceptor, which is incapable of causing dissolution of cells without the first, hence the term complement is applied to it. Complement is normally present in all sera freshly drawn from the body, but disappears gradually on standing or is completely destroyed at from 55° to 56°C. in about thirty minutes. Complement of one species is not identical in its action with that of other species.

Complement deflection.

Synonymous with complement deviation.

Complement deviation.

Synonymous with deflection; originates from a German term Ablenkung, introduced by Neisser. Complement deviation is identical with Komplementablenkung of the Germans, and fixation of alexin of the French. By the deviation of complement one understands that complement is fixed by the antigen-antibody combination and is made unavailable for a second set of antigen-antibody combination to complete a reaction in which complement is essential. This second set may be a hæmolytic or a bacteriolytic system. See illustrations on pages 24 and 25.

Complement fixation.

Synonymous with complement deviation.

Complementoids.

Modified complements in which the zymotoxic group is destroyed without losing their binding property with amboceptors. Complementoids are formed at 56°C.

Complementophilic group.

Atom-complex of amboceptor on which complement anchors. This complex remains inactive until the cytophilic group (another atom-complex) of the amboceptor joins with the receptor of the cell.

Copula.

Synonymous with amboceptor.

Cytase.

Introduced by Metchnikoff; is synonymous with alexin of Bordet and complement of Ehrlich. See Complement.

Cytolysins.

Active substances in blood serum consisting of specific cytolytic amboceptors and complement.

Cytolysis.

Dissolution of cells by specific amboceptors and complement. In case of blood corpuscles the term hæmolysis is used and for bacteria the term bacteriolysis is used.

Cytophilic group.

Atom-complex of amboceptor with which the receptor of a cell combines. Thus an amboceptor possesses two atom-complexes, one for the complement and the other for the receptor of the cell.

Endotoxin.

Toxic constituents of bacterial cells.

Fixateur.

Metchnikoff's term for Amboceptor of Ehrlich and Substance sensibilisatrice of Bordet. See Amboceptor.

Hæmolysins.

Any substance capable of causing hæmolysis may be called an hæmolysin, but its use is restricted to the biological products of unknown chemical constitution, especially the blood serum, or often the amboceptor of the serum.

Hæmolysis.

Dissolution of blood-corpuscles by various forces, setting the hæmoglobin free into the medium in which the corpuscles are suspended. Distilled water, freezing and thawing, temperature of about 55°C. for 30 minutes, etc., are physical agents which cause hæmolysis. Acids, alkalies, and certain salts can cause hæmolysis in proper concentrations. Of these chemicals may be mentioned most organic acids, mineral acids, all alkalies, bile salts, bichloride of mercury, soaps. Of biological origin may be mentioned certain glycosides such as saponin, solanin, etc., certain bacterial cultures such as those of staphylococcus, vibrios, megatherium, tetanus bacillus, etc.; certain animal venoms such as those of snakes, bees, spiders, etc. The hæmolytic process caused by these different agents is different according to the nature of the hæmolytic forces, but they attack the corpuscles more or less directly. Hæmolysis by serum is, however, somewhat different from that caused by the various forces just mentioned. Thus, hæmolysis by fresh alien serum is caused by two distinct groups of substances both contained in blood serum. One is called complement and the other amboceptor. The one is inactive without the other. Serum hæmolysis forms the basis of many interesting phenomena, the serum diagnosis of syphilis being one of these.

Hæmolytic amboceptors.

See under Amboceptors.

Haptins.

Introduced by Ehrlich; synonymous with antibodies, except in somewbat broader sense.

Haptophore group.

The atom-complex of complement which has the power of anchoring on the complementophilic group of amboceptor, thus uniting complement with the cell through tbe intermediation of amboceptor.

Immune bodies.

Synonymous with antibodies.

Inactivation.

Fresh serum containing both amboceptor and complement, becomes inactive when beated to from 55° to 56°C. for about 30 minutes because of tbe destruction of complement. This process is called inactivation, and the heated serum is called inactivated serum. Amboceptor is not affected materially by the process.

Inter-body.

Ebrlich used the term Zwiscbenkörper before he introduced tbe term Amboceptor, and its English version is Inter-body (Bolduan) or Intermediary body (Flexner and Noguebi).

Intermediary body.

Synonymous with Inter-body; an English translation of Ehrlicb's Zwischenkörper. Identical with amboceptor.

Iso-agglutinin.

Blood serum of an animal usually does not contain agglutinins for the blood-corpuscles of another animal of the same species, but in some instances agglutination may occur and is due to the substances called iso-agglutinins.

Iso-hæmolysin.

Blood serum of one animal usually does not hæmolyse the blood-corpuscles of another animal of tbe same species, but in some instances hæmolysis may occur. This phenomenon is known as isohæmolysis and is caused by the presence of iso-hæmolysin. In man this is observed quite frequently in the serum of patients suffering from malignant tumors.

Komplementablenkung.

Synonymous with complement deviation.

Komplementbindung.

Synonymous wtih complement deviation.

Komplementverankelung.

Synonymous with complement deviation.

Komplementoidverstopfung.

Prevention of complement fixation on account of interference on the part of complementoid. In case of hæmolysis, this causes inhibition of hæmolysis.

Opsonins.

Introduced by A. E. Wright; are active substances of normal as well as immune sera causing phagocytosis. Normal opsonins are rendered inactive at 56°C. and seem to depend upon the coöperation of complement. Immune opsonins are thermostable.

Pleocytosis.

Introduced by Nonne and identical with lymphocytosis in the cerebrospinal fluid in syphilitic and parasyphilitic diseases of the central nervous system.

Precipitates.

By the term precipitate in immunity work is meant the flocculence or clumps formed by mixing specific antigen and antibody, such as serum precipitates, bacterial extract precipitates, etc.

Precipitation.

In immunity work one understands by precipitation a clumping phenomenon of protein or protein-like substances by specific precipitins.

Precipitin.

In the blood serum of an animal which received repeated injections of a solution of proteid matter there is found a substance capable of precipitating that proteid when mixed in a test tube. This precipitating principle is called precipitin, and its action is specific; that is, a precipitin for human serum precipitates the latter, but no other serum. Precipitins can be produced in animals for different proteins, such as egg albumen, serum, milk, bacterial proteins, etc. It is resistant to the temperature of 56°C. like most immunization products and remains active for a very long time when desiccated.

Precipitinogen.

A general term occasionally used for the substances capable of producing precipitins by means of immunization, or repeated injections into animals.

Preparator.

Synonymous with Amboceptor.

Protectin.

A term introduced by Noguchi to designate a substance (or substances) developing in all blood sera on standing *in vitro*, and characterized by its or their effects in protecting blood-corpuscles against hæmolytic serum. This protective substance (or substances) is taken up by the corpuscles through long contact, this property being increased in sera of

certain animals after heating to 60°C. or a little higher. It is similar to the complementoid of Ehrlich and Morgenroth, differing only in its absorbability by non-sensitized cells and extractability by fat solvents such as ether, acetone, and alcohol.

Reactivation.

The addition of complement to an inactivated serum restores its lytic activity, and the process is called reactivation.

Receptors.

Constituents of the cell uniting with amboceptors or any other antibodies or toxins. The presence or absence of receptors determines whether the cell is susceptible to a given amboceptor or toxin, or not.

Sensibilisation.

Synonymous with sensitization and is caused by allowing amboceptor to act on cells.

Sensitization.

When a cell is acted upon by specific amboceptor it becomes sensitive to the dissolving action of complement. This process of rendering a cell sensitive is called sensitization. In French it is sensibilisation.

Sensitizer.

Synonymous with Amboceptor of Ehrlich and Bordet's Substance sensibilisatrice.

Sensitizing substance.

Synonymous with Amboceptor.

Stimulin.

Introduced by Metchnikoff; is an active principle of serum favoring phagocytosis. Metchnikoff thought it due to the stimulation of phagocytes but it is probably identical with the opsonins of Wright.

Substance sensibilisatrice.

Introduced by Bordet; is synonymous with Ehrlich's Amboceptor, Metchnikoff's Fixateur, Gruber's Preparator, or P. Th. Müller's Copula. Bordet often uses the term "sensibilisatrice." See Amboceptor.

Thermolabile.

Complement loses its activity at about 55° to 56°C. in about 30 minutes and is called thermolabile.

Thermostable.

Amboceptor remains still active after the serum containing it is heated at 55° to 56°C. for 30 minutes, and is said to be thermostable.

Toxins.

A general term for a group of substances mostly of bacterial elaboration, possessing a powerful toxicity and one or more of the following characteristics. Thermolability, incubation period for action, unknown chemical constitution, difficulty in separating from protein molecule, capability of producing antibodies. Best known examples are diphtheria toxin and tetanus toxin, both of which belong to true toxins and are of extracellular origin. The toxic principles of cholera vibrio, meningococcus, gonoccocus, typhoid bacillus, dysentery bacillus are chiefly contained in the cell-body and are called endotoxins. Tuberculin is an atypical, extracellular toxin. Toxalbumins of higher plants and toxic secretions of snakes, spiders, and bees resemble bacterial toxins in many respects.

Toxoids.

Ehrlich modified various toxins by chemicals in such a manner as to reduce or remove their toxic property without destroying their immunizing property. They may arise spontaneously under certain circumstances and combine, like toxins, with antitoxins. The modified toxins are called toxoids.

Toxophore group.

Analogous with zymotoxic group of complement.

Zymotoxic group.

Analogous with toxophore group of a toxin and represents the active, dissolving atom-complex of a complement. The destruction of this group leads to the formation of a complementoid.

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